METHODS FOR IDENTIFYING NOVEL MULTIMERIC AGENTS THAT MODULATE RECEPTORS

Substitute Specification and Drawings

Application No.: 10/015,534
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CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Application Serial No. 60/092,938, filed July 15, 1998 and U.S. Provisional Application Serial No. 60/088,466, filed June 8, 1998, both of which are incorporated herein in their

10   entirety by reference.

BACKGROUND OF THE INVENTION

Field of the Invention

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This invention is directed to general synthetic methods for generating large libraries of diverse multimeric compounds capable of binding cellular receptors which multimeric compounds are candidates for possessing multibinding properties. The diverse multimeric compound libraries provided by this invention

20   are synthesized by combining a linker or linkers with a ligand or ligands to provide for a library of multimeric compounds wherein the linker and ligand each have complementary functional groups permitting covalent linkage. The library of linkers is preferably selected to have diverse properties such as valency, linker length, linker geometry and rigidity, hydrophilicity or hydrophobicity,

25   amphiphilicity, acidity, basicity, polarizability and polarization. The library of ligands is preferably selected to have diverse attachment points on the same ligand, different functional groups at the same site of otherwise the same ligand, and the like.

This invention also relates to ligands which bind to receptors and modulate their activity in living systems. More particularly, the invention relates to novel compounds that bind to and modulate the activity of receptors by acting as multi-binding agents. The multi-binding agents of the invention comprise at least two ligands connected by a linker or linkers, wherein said ligands in their monovalent state bind to and/or are capable of modulating the activity of the receptor. The linking moiety is chosen such that the multi-binding agents so constructed demonstrate increased biological activity as compared to individual units of the ligand. The invention is also related to methods of using such compounds, to methods of preparing such compounds and to pharmaceutical compositions containing them.

These multi-binding compounds are particularly useful in treating conditions in a mammal that are mediated by the cellular receptors targeted by the ligands. Accordingly, this invention also relates to pharmaceutical compositions comprising a pharmaceutically acceptable excipient and an effective amount of a compound of this invention.

These multi-binding compounds may also be used as insecticides, and for other argricultural applications such as crop protection. Additionally, they are useful as affinity resins for affinity chromatography.

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All of the above publications, patent applications and patents are herein incorporated by reference in their entirety to the same extent as if each individual publication, patent application or patent was specifically and individually indicated to be incorporated by reference in its entirety.

State of the Art

A receptor is a biological structure with one or more binding domains that reversibly complexes with one or more ligands, where that complexation has biological consequences. Receptors are distinguished for the purpose of this application from enzymes, which bind and then transform the bound species. Receptors are most often proteins.

Receptors can exist entirely outside the cell (extracellularly), within the cell membrane (but presenting sections of the receptor to the extracellular milieu and cytosol), or entirely within the cell (intracellularly). They may also function

independently of a cell (e.g. clot formation). Receptors within the cell membrane allow a cell to communicate with the space outside of its boundaries (i.e. signaling) as well as function in the transport of molecules and ions into and out of the cell.

A ligand is a binding partner for a receptor. A ligand may be the endogenous ligand for the receptor or alternatively may be a synthetic ligand for the receptor such as a drug, a drug candidate or a pharmacological tool.

Receptors can be categorized as G-protein coupled receptors, tyrosine kinase linked receptors, guanylate-cyclase linked receptors, nuclear steroid receptors, membrane bound steroid receptors, ligand-gated ion channel receptors and adhesion molecules.

1. G-protein coupled receptors

The super family of seven transmembrane proteins (7-TMs), also called G-protein coupled receptors (GPCRs), represents one of the most significant classes of membrane bound receptors that communicates changes that occur outside of the cell's boundaries to its interior, triggering a cellular response when appropriate. The G-proteins when activated, affect both positively and negatively a wide range of downstream effector systems (e.g. ion channels, protein kinase cascades, transcription, transmigration of adhesion proteins).

The GPCR is a membrane bound cell surface receptor that is comprised of a single polypeptide chain. It is composed of seven hydrophobic transmembrane helices. The N-terminus is extracellular, the C-terminus is intracellular. The sequential three dimensional orientation of these helices provides a number of binding domains for a variety of endogenous ligands, and the G-proteins. These molecules bind to and modulate the functional activity of these receptors.

One remarkable feature of a number of the 7-TM receptor classes is their widespread distribution and the different disease states that can be ameliorated by creation of subtype specific ligands. Examples include members of the

serotonergic class (currently used with migraine therapy, GI motility agents, antipsychotics and antidepressants) and members of the adrenergic class (useful in the treatment of hypertension, asthma, prostrate disease and depression).

There are different classes of 7-TM receptors. Examples of ligands which bind to receptors include the following:

Receptor	Current and Potential Therapeutic Indication(s)	Drugs/Ligands
ACTH releasing factor (CRF)	Anxiety disorder, Depression (Obsessive/compulsive disorder, Cerebrovascular ischemia, Gastrointestinal disease, Alzheimers disease, Dementia, Obesity, Anorexia nervosa, Central nervous system disease, Behavior disorder, Thrombosis)	NBI-103, NBI-30775, NBI-104, NBI-27914, NBI-29356, SG-058/SC-241, PD-171729, CRA-0165, ebiratide, SA-627, SD-491, JE-884, Org-2766, CP-154526, NBI-31199, NBI-31200, NBI-112, CRA-1000, CRA-1001, NBI-104, NBI-30545, MCI-028, SC-241, NGD-98-1, DMP-695, urocortin, SG-058, Org-31433, SJ-948, NBI-113
Adenosine A1	Hypertension, Renal disease (Diuresis, Hypertension, Cognitive disorder, Renal failure, Heart arrhythmia, Cardiovascular disease, Allergy, Asthma, Inflammation, Heart disease, Depression, Cardiac failure, Angina). Alzheimer's disease	FK-352, KW-3902, SDZ-WAG-994, NNC-210136, CVT-510, FR-166124, PD-81723, IRFI-117, WRC-0571, UP-20256, N-0840, KF-15372, N-0861, KW-3902, CVT-124, GR-79236, KF-17837, midaxifylline, NNC-21-0041, NNC-90-1515, methoxy flavone derivatives, apaxifylline, FK-453, AMP-579, NNC-21-0091, NNC-21-0238, RG-14202, N-0723, UP-2-2-56, FK-838, PD-126280, N-0840, BN-063, IRFI-165, KF-15372, BW-1205U90, MDL-102503, DTI-0009, WRC-0571, UP-202-32, GP-04012, RS-74513-000, Sch-59761

Receptor	Current and Potential Therapeutic Indication(s)	Drugs/Ligands
Adenosine A2	Parkinsons disease (Depression, Thromboembolism, Neurodegenerative disease, Neurological disease, Anxiety disorder, Pain), Hypertension, Restenosis (Cardiovascular disease, Asthma, Hypotension)	KW-6002, KF-17867, WRC-0470, SEP-89068/SEP-119249, Sch-58261/8FB-PTP, CGS-21680C, methoxy flavone derivatives, AMP-579, ZM-241385, 2-(3-cyclopentyl-1-propyn-1-yl)adenosine, HENECA, 8FB-PTP, CGS-22989, YT-146, CGS-15943, MDL-101483, GW-328267, Sch-59761, KF-17867, CL-356381, CL-288875
Adrenergic α1a	Benign Prostatic Hypertrophy	Tamsulosin, SNAP-5089, SNAP-5399, SNAP-5582, SNAP-6107, SNAP-6145, SNAP-6201, SNAP-6262, SNAP-7600, SNAP-6719, SNAP-7461, SNAP-7443, SNAP-7555, SNAP-7556, SNAP-7180, SNAP-7292, SNAP-5540, naftopidil, terazosin, RS-100975, A-131701, SB-216469, KMD-3213, SL-890591, doxazosin, prazosin, alfuzosin, L-794191, L-757464, trimazosin
Adrenergic β3	Obesity, Irritable bowel syndrome, NIDDM	L-362884, SR-59230A, ICI-198157, SR-58611A, L-749372, CP-114271, AD-9677, SM-11044, L-755507, SB-226552, CL-316243, BRL-37344, BMS-196085, CGP-12177, LY-362884, BMS-187257, ICI-201651

Receptor	Current and Potential Therapeutic Indication(s)	Drugs/Ligands
Angiotensin II	Hypertension, CHF	Losartan, valsartan, irbesartan, candesartan, eprosartan, tasosartan, telmisartan, ripisartan, CS-866, DA-727, KRH-594, LR-B/081, TAK-536, YM-358, MK-996, milfasartan, BIBS-39, KT3-671, L-162313, BIBS-222, CL-329167, zolasartan, EXP-597, DuP-532, EXP-332, PD-123319, PD-121981, SC-52458, L-163579, L-163017, A-81988, XH-148, EMD-66684, ME-3221, L-163007, L-159689, L-162234, L-159913, UR-7198, L-162389, L-162638, PD-126055, S-0029, L-162686, RU-64276, CGP-42112, BIBR-363, XR-510, L-163598, L-162393, SB-203220, EXP-408, KW-3433, KRH-594, WK-1492, L-162620, EXP-970, YM-31472, PD-123177, TAK-536, L-158809, L-158978, HR-720, L-735286, WAY-126227, KT3-866, SC054629, RWJ-47639, BMS-183920, LF7-0156, LY-301875, L-161290, L-162132, EXP-801, RU-65868, ICI-D-8731, GR-137977, SK-1080, DMP-581, A-81282, CI-996, FR-149581, 606A,
Bradykinin BK-2	Asthma, Pain, Pancreatitis, Cardiovascular disease, Urinary tract disorders, Cerebrovascular ischemia, Allergy, Inflammation, Arthritis	FR-167344, NPC-17731, CP-0597, icatibant, NPC-567, WIN-64388, CP-0578, FR-190997, B-9340, NPC-18884, LF-160335, FR-184280, B-9430, B-9698, CP-2559, B-9858, CP-2550, NPC-16731, MEN-11270, FR-172357, S-16118, FK-3657, LF-16-0687, NPC-17761, CP-0364, CP-840, CP-0719, CP-2055, NPC-18521, NPC-18688, PS-322835
Cannabinoid	Emesis, AIDS-related complex, Alzheimers disease, Anorexia nervosa, Depression, Sustance dependence, Allergy, Inflammation, Pulmonary disease, Immune disorder, Glaucoma, Psychosis, Pain, Gastrointestinal disease, Urinary tract disease, Cardiovascualar disease, Brain injury, Cognitive disorder	Dronabinol, P-0792, SR-141716, WIN-55212-3, HU-210, L-759787/L-768242, DP-55940, BAY-38-7271, WIN-55212-2, morpholinoalkylindenes, anandamide, palmidrol, JWH-015, SR-144528, WIN-56098, SR-140098, AM-630, SR-41716A, O-585, JWH-051, WIN-54461, L-759633, L-759656, CP-55940, O-823, O-689, LY-320135, CP-55244, CT-3, nabilone, P-0792

Receptor	Current and Potential Therapeutic Indication(s)	Drugs/Ligands
Chemokine CXCR-3	Inflammation	SKF-83589, SB-225002
Chemokine CCR-3	asthma, psoriasis, multiple sclerosis, rheumatoid arthritis	Chemotides, NSC-651016, NSC-645797, NSC-655720
CGRP	Migraine, Non-insulin dependent diabetes, Inflammation	Capsinolol, BIBN-4096
Cholecystokinin A (CCK A)	Carcinoma, Eating disorder, Gastric motility disorder, Gastrointestinal disease, Pancreatitis, Pain, Obesity, Anxiety disorder, Schizophrenia, Motility disorder, Gall bladder disease	Loxiglumide, dexloxiglumide, FK-480, TP-680, GW-5823, PD-149164, PD-145942, SR-146131, T-0632, FPL-15849, FR-193108, A-71623, loxiglumide, lintitript, IQM-95333, GW-7854, GW-7178, A-74498, A-71623, A-71378, PD-142898, FR-208419, A-70276, FPL-15849, dexoxiglumide, PD-140548, FPL-14294, A-70874, A-57282, devazepide, A-68552, PD-151932, PD-149164, Z-203, SR-146131, A-71378, SR-27950, T-0632, KSG-504, TP-680, GI-248573, L-365031
Cholecystokinin B (CCK B, Gastrin receptor)	Sleep disorder, Anxiety disorder, Drug dependence, Gastrointestinal disease, Neurological, mental, and cognitive disorders, Pain, Pancreatitis, Depression, Central nervous system disease, Peptic ulcer, Anorexia nervosa, Eating disorder, Substance dependence	GV-150013, L-740093, JB-931182, PD-135158, PD-145942, L-368935, L-369466, L-736380, AG-041R, Tetronothiodin, RPR-101367, YM-220, FR-193108, GV-150013, PD-134308, tetronothiodin, YM-022, L-708474, CI-988, GW-7854, L-365260, C1015, L-736380, GR-199114X, L-751892, PD-142898, RPR-107836, CP-212454, JB-931182, FK-480, CR-2622, CR-2194, PD-136450, FR-208419, L-369466, Ro-09-1540, DZ-3514, L-708474, RP-69758, CR-2345, CR-2945, RPR-102681, GV-191869, lorglumide, CR-2194, LY-288513, FR-175985, PD-135666, RP-72540, RP-71483, LY-262291, LY-, YF-476, L-156586, RP-73870, D-15-9927, D-61-1608

Receptor	Current and Potential Therapeutic Indication(s)	Drugs/Ligands
Endothelin	Pulmonary Hypertension, CHF, Hypertension, Pancreatitis, Renal failure, Migraine, Restenosis, Myocardial infarction, Atherosclerosis, Glaucoma	Bosantan, PD-145065, Ro-46-2005, BQ-610, SB-209670, BQ-123, TAK-044, FR-139317, BMS-182874, TBC-11251, BQ-153, PD-161721, PD-156707, PD-142893, PD-155080, CGS-27830, PD-155218, FR-901533, L-744453, LU-135252, PD-151242, PD-160874, EMD-122801, IRL-1666, BQ-3020, ZD-1611, S-0139, ABT-627, T-0115, RPR-111844, YM-598, L-749239, FR-901367, PD-156252, LU-302872, A-104029, BQ-928, PD-159433, PD-162073, SQ-35469, SQ-34520, J-104132, IRL-3461, BMS-207940, A-182086, TAN-2162, IRL-1543, A-158112, A-207508, IPI-616, TBC-2576, PD-152884, PD-156123, 97-139, LU-127043, A-104029, L-746072, VML-588, T-0201, L-747844, EMD-122946, Ro-485695, SB-234551, PD-102566, RES-701-2, RES-1214-1, PD-159110, PD-160672, PD-159020, RPR-105227, RPR-111613, Ro-43-1736, A-216546, ATZ-1993, ZD-2574, LU-302146, ABT-147627, BQ-145, stachybosins, BMS-187308, A-201661, PD-166114, EMD-94246, BQ-518, TBC-3214, TBC-11299, RPR-110477, TBC-11040, Ro-61-0612, ZD-4054, BMS-193884, Ro-48-5694, S-1265, SB-255757, PD-166673, SB-215355, PD-180988, PD-163070, TBC-10662, Ro-44-9099, Ro-06-2687, BQ-485, 50-235, BQ-238, J-104121, IRL-2500, IRL-2659, IRL-1038, IRL-2796, PD-155719, SB-247083, PD-163610, BE-18257B,
EP 2-4	Myocardial infarction (Pain, Inflammation); agonist	ONO-AE-248, ONO-NT012, GR-63799, MB-28767
Galanin	Obesity, Cognitive disorder	Sch-202596
Glucagon	Diabetes mellitus (Insulin dependent diabetes, Non-insulin dependent diabetes, Acidosis, Obesity)	L-168049, BAY-27-9955, NNC-92-1687, ALT-3000, A-4166, L-168049, CP-99-711

Receptor	Current and Potential Therapeutic Indication(s)	Drugs/Ligands
Metabotropic glutamate 1	Epilepsy, Cerebrovascular ischemia, Head injury, Alzheimers disease, Ischemia, Pain	NNC-07-0775, 3,5-DHPG, NPS-2390, ACPD, UPF-523, LY-393675, LY-3390334, UPF-596, LY-367385, LY-302427, ACUDA
Metabotropic glutamate 2	Anxiety disorder, Nicotine use disorder, Central nervous system disease, Substance dependence, Epilepsy, Neurodegenerative disease	LY-354740, F-2-CCG-I, NNC-07-0775, LY-341495
Histamine H1	Allergy, Asthma, Eczema, Papular skin disease, Urticaria, Pruritis, Rhinitis, Ocular disease	Loratidine, carebastine, norastemizole, efletirizine, HSR-609, emedastine, terfenadine, ZCR-2060, WY-49051, KAA-276, epinastine, ebastine, E-4716, KC-11404, fexofenadine, FK-613, selenotifan, DF-1111301, loratidine, MDL-28163, MDL-103896, desloratidine, mizolastine, KC-11425, KA-398, cetirizine, noberastine, VUF-L-9015, tagorizine, histaprodifen, alinastine, diphenhydramine, , chlorpheniramine, ketotifen, oxatomide, astemizole, acrivastine, azelastine, triprolidine, hydroxyzine, azatadine, rupatadine, MDL-16455, pyrilamine, promethazine, chlorcyclizine, carbinoxamine, clemastine, dimenhydrinate, tripelennamine, brompheniramine, cyclizine, meclizine, levocabastine
Histamine H2	Duodenal ulcer, Gastritis, Stomach ulcer (Peptic ulcer, Ulcer)	Famotidine, ebrotidine, lafutidine, osutidine, IT-066, P-Z-300, KP-105, BMY-25368 , IGN-2098, osutidine, FRG-8701, Z-300, KP-105, IY-80845, cimetidine, ranitidine, famotidine, nizatidine

Receptor	Current and Potential Therapeutic Indication(s)	Drugs/Ligands
Histamine H3	Cognitive disorders (Alzheimers disease, Depression, Epilepsy, Obesity, Sleep disorder, Attention deficit hyperactivity disorder, Eating disorder, Central nervous system disease, Neurological disease, Allergy, Asthma, Mental disorder)	GR-175737; GT-2331, UCL-1972, GR-168320, GT-2016, AQ-0145, UCL-1199, GR-175737, verongamine, BP-294, FUB-181, ciproxifan, GR-168320, thioperamide, VUF-9153, FUB-181, UCL-1972, proxyfan, iodoproxyfan, UCL-1199, VUF-4163, SKF-91606, imifluramine, BP-294, UCL-1283, GT-2104, GT-2203, impentamine, UCL-1409, UCL-1390
5-HT 2	Psychosis, Schizrenia, Depression, Sexual dysfunction, Anxiety, Sleep disorders, Migraine, Cardiovascular disease, Thromboembolism, Hypertension	Risperidone, dotarizine, metergoline, sergolexole, fananserin, adatanserin, ritanserin, amesergide, iloperidone, SR-46349, carvotroline, perospirone, SM-13496, sertindole, ziprasidone, YM-35992, deramciclane, olanzapine, dotarizine, ocaperidone, amperozide, Org-5222, CV-5197, mirtazapine, AD-5423, flibanserin, zotepine, LU-29066, P-1704, DV-7028, quetiapine, LU-26042, LEK-8829, glemanserin, ketanserin, ICI-170809, SR-46615A, CGS-18102A, pelanserin, NNC-22-0031, LY-215840, E-2040, BMS-181102, SUN-9221, NPC-18166, P-706A, SB-224289, ZD-3638, SDZ-MAR-327, NRA-0045, MDL-28133A, perbufylline, nefazodone, DuP-734, SUN-C-5174, balaperidone, ICI-169369, lintopride, P-9662, HT-90B, FG-5803, BW-1205U90, EX-711, metergoline, lidanserin, sarpogrelate, AT-1015, alentemol, emopamil, LY-053857, JL-13

Receptor	Current and Potential Therapeutic Indication(s)	Drugs/Ligands
5-HT 4	Constipation, Gastric motility disorder, Irritable bowel syndrome, IBD, Pain, Gastrointestinal disease, Neurological disease, Emesis, Dyspepsia, Esophagitis, Central nervous system disease, migraine, anxiety disorder	Piboserod, tegaserod, lintopride, renzapride, mosapride, itasetron, fabesetron, SB-207266, SB-207710, SB-204070, SB-203186, SDZ-205-557, R-93877, E-3620, YM-47813, SL-65.0102, LY-297524, LY-353433, RS-39604, RS-33800, RS-67506, RS-67333, RS-17017, RS-23597-190, RS-16566, RS-67532, RS-56532, RS-100235, BIMU-8, KGA-0941, SC-55387, Y-36912, SDZ-216454, SDZ-205-557, SC-53116, SC-52491, SC-53606, SC-50410, GR-113808, FCE-29029, FCE-29034, ML-1035, KGA-0941, SK-951, GR-125487
Leukotriene D4	Asthma, inflammation	Zafirlukast, tomelukast, montelukast, pobilukast, CGP-44826, CGP-57698, L-695499, L-708734, L-708738, L-648051, L-733321, LY-287192, LY-290154, SKF-106203, LM-1453, CP-199330, OT-4003, FK-011, MCI-826, RG-12525, CP-195494, FPL-55712
Neuropeptide Y Receptor	Bulimia nervosa, Eating disorder, Obesity, Hypertension, Depression, Heart disease	NGD-95-1, SR-120819A, SR-120107A, GW-1229, CGP-71683A, BW-1229, GI-264879A, BMS-192548, SNAP-6608, BIBP-3226, PD-160170, BIBO-3304, SNX-024

Receptor	Current and Potential Therapeutic Indication(s)	Drugs/Ligands
Platelet Activating Receptor (PAF)	Asthma, Conjunctivitis, Coronary artery disease, Multiple sclerosis, Pancreatitis, Sepsis (Inflammatory bowel disease, Pneumonia, Respiratory distress syndrome, Reperfusion injury, Colitis, Insulin dependent diabetes, Disseminated intravascular coagulation, Thromboembolism, Ulcerative colitis, Endotoxic shock, Carcinoma, Leukemia, Thrombocytosis, Thrombocytopenia, Pain, Myocardial infarction, Pneumonia, Shock, HIV infection, Cerebrovascular ischemia, Ischemic heart disease, Immune disorder, Cardiovascular disease, Sepsis, Rhinitis)	Lexipafant, YM-461, UK-91473, ZG-1494-alpha, Sch-37370, SR-27417, BN-50739, TCV-309, CV-6209, Y-24180m, CP-96486, CP-96021, ABT-299, LM-3370, KC-11404, rocepafant, Rupatidine, SDZ-62-434, apafant, UR-12633, selenotifen, ginkgolide B
Substance P (Neurokinin 1, NK 1)	Asthma, inflammation, psoriasis, arthritis, IBD, Emesis, Pain, migraine, anxiety, depression, psychosis	MK-0869, FR-113680, L-737488, CGP-49823, CP-122721, FK-888, GR-82334, GR-203040, CP-96345, CP-99994, MEN-10930, SR-140333, dapitant, lanepitant, RP-67580, L-742694, L-741671, L-758298, L-733060, YM-49244, S-16474, S-18523, MDL-105212, LY-306740
Vasopressin V1	Cardiac failure, Hypertension, Nephrotoxicity, Cardiovascular disease, Diuresis, Dysmenorrhea, Emesis, edema	OPC-21268, YM-087, SR-49059, VPA-985, CL-385004, YM-471, F-180, JTV-605

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Receptor	Current and Potential Therapeutic Indication(s)	Drugs/Ligands
Vasopressin V2	Hypertension, Cardiac failure, Renal failure, NIDDM	OPC-31260, YM-087, VPA-985, CL-385004, YM-471, FR-161282, SR-121463, WAY-140288, OPC-41061, SKF-101926, CL-385004
Adrenergic α2	Anesthesia, Multiple sclerosis, Muscle hypertonia, Muscular spasm, Ocular hypertension, Glaucoma, Pain, Hypertension, Cognitive disorders, Migraine, Central nervous system disease, Attention deficit hyperactivity disorder, Sedation, Diabetes mellitus, Cardiovascular disease, Micturition disorder, Thromboembolism, Impotence, Sexual dysfunction	Dexmedetomidine, mivazerol, idazoxan, fluparoxan, AGN-193080/AGN-192172, AGN-190837, SKF-104078/BRL-44408, L-657743, F-10981, PGE-6201204, UK-1403, fluparoxan, brimonidine, ecabapide, CHF-1035, S-17089-1, AGN-192836, AGN-191103, midodrine, UK-14304, amosualol, indoramin, PMS-812, TA-993, delequamine
Chemokine CCR-5, CXCR-4	HIV infection, viral infection	VMIP-II, AOP-RANTES, Nananoyl-RANTES, SDF-1, NSC-651016, NSC-645797, NSC-655720, AMD-3100, T-22, SPC-3

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There is also a breadth of diversity within the ligands that modulate the downstream signaling activity of the 7-TM receptors. These ligands range from the small biogenic amines (serotonin, histamine, dopamine), lipids (prostaglandins and the endogenous cannabinoids), neuropeptides (neurokinins, NPY, opioids),

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peptide hormones (angiotensin and bradykinin) to larger peptides such as chemokines and thrombin. Pharmacological studies employing mutational and chimeric receptor constructs have defined the binding domains for this diverse library of ligands. These studies have provided some fundamental principles for defining the ligand binding domains of these receptors. 7-TMs have been

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categorized based on the binding interactions of the endogenous ligands.

Family 1a   Rhodopsin family, olfactory, catecholamines, opioid

Family 1b   Peptides, cytokines, thrombin

Family 1c   Glycoprotein hormones e.g. LH, TSH, FSH, CG

Family 2   Secretin Family of calcitonin like receptors, e.g. PCAP, glucagon, CRF, VIP

Family 3   Metabotropic glutamate receptors e.g. mGluR1-5

2. Ligand-gated Ion Channel receptors

Another family of receptors is the ligand-gated ion channel receptors. Here the binding of ligands to specific ligand binding sites on these receptors results in modulation of ion flux into or out of the cell. These membrane bound cell surface receptors are composed of multiple subunits, typically 5 subunits, which may be the same of different.

These ligand-gated ion channel receptors have important roles in the central nervous system and peripheral nervous system. Examples of the receptors include the $GABA_A$ receptor, the NMDA receptor, 5-HT-3 receptor and nicotinic acetylcholine receptor. Examples of the ligands which bind to these receptors and their therapeutic indications include the following:

Receptor	Current and Potential Therapeutic Indication(s)	Drugs/Ligands
GABA A	Anxiety, sleep disorder, cerebrovascular ischemia, epilepsy, psychosis, schizophrenia, Alzheimer=s disease	Diazepam, ganaxolone, clomethiazole, suritozole, imidazenil, losigamone, pagoclone, RY-024, AHR-14042, RWJ-38293, SB-205384, Co-60549, Co-2-6749, Co-122739, Co-2-1068, CCD-3693, RWJ-53050, RWJ-46771, PF-1885, U-104841, U-93631, U-101017, Co-152791, S-8510, RU-33965, RU-5135, MDL-27192, BRL-54504AX, AWD-131-138, PNU-107484A, PNU-100076, NS-2710, NC-1201, FG-8094, NGD-91-2

Receptor	Current and Potential Therapeutic Indication(s)	Drugs/Ligands
5-HT 3	Emesis, anxiety disorder, cognitive disorders, dementia, depression, schizophrenia, gastric motility disorder, nausea, irritable bowel syndrome, heart arrhythmia, drug dependence	Ondansetron, lurosetron, azasetron, itasetron, fabesetron, alosetron, ramosetron, dolasetron, mirtazapine, palonosetron, cilansetron, Granisetron, renzapride, R-zacopride, zatosetron, Tropisetron, lerisetron, ricasetron, YM-114, CP-93318, N-3256, WAY-100289, SC-50410, KF-18259, RS-56812, SC-52491, E-3620, SC-52246, SDZ-ICM-567, UCM-30593, GR-65630, DAT-582, ADR-851, N-3389, RS-33800, RS-56532, KB-6806, KGA-0941, S-21007, LY-278584, DAU-6285, R-093777, GYKI-46903, ML-1035, L-683877, KB-6933, RG-12915, SC-52150, GK-128
NMDA	Pain, neurological disease, neuroprotective agent, cerebral infarction, cerebrovascular ischemia, cerebral hypoxia, schizophrenia, epilepsy, head injury, Parkinson=s disease, drug dependence	Ifenprodil, dexanabinol, midafotel, remacemide, eliprodil, Ro-24-6173, Ro-24-6449, Ro-8-4304, GPI-3000, ARL-15896AR, ADCI, FPL-16283, LY-274614, WAY-126090, HO-473, CNS-1531, selfotel, CP-101606, CP-98113, ES-2421, BI-II-277-CL, FR-115427, CNS-5161, CNS-1044, CNS-5065, CNS-1118, CNS-1524, CNS-1505, L-701315, L-701376, L-701252, L-698532, L-689560, L-687414, L-701273, LY-235959, LY-233053, LY-235723, LY-233536, EMD-95885, CGP-39653, CGP-37849, MRZ-2/579, HA-966, CP-101616, AP-6, NC-1210, PD-158473, NPS-1506
Nicotinic Acetylcholine	Pain, Alzheimer=s disease, cognitive disorders, muscle spasm, obesity	ABT-594, ABT-089, GTS-21, RJR-2403, RJR-1401, RJR-2429, RJR-1734, A-85380, A-82695, SIB-1553A, SIB-1508Y, SIB-1765F, ANQ-9040, S-1663, UB-165, DBO-83, anorectic

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3.   Tyrosine Kinase linked and guanylate cyclase linked receptors

Tyrosine kinase linked receptors mediate the actions of a number of peptide mediators (eg. insulin and other growth factors). The receptors for most growth factors are transmembrane tyrosine kinase receptors, including receptors for platelet-derived growth factor (PDGF), fibroblast growth factors (FGFs), EGF, TGF, hergulin, insulin, insulin-like growth factors (IGF) I and II, nerve growth

factor, stem cell factor, vascular endothelial growth factor, macrophage colony stimulating factors (CSF) and others.

The receptors for growth hormone, prolactin, erythropoietin, IL-2, IL-3, IL-4, IL-6, IL-7 granulocyte CSF, granulocyte-macrophage CSF, interferon, interferon, ciliary neurotrophic factor and many other cytokines are members of the tyrosine kinase-associated receptor family. These receptors are often over-expressed on tumors of hematopoietic origin and similar to receptor tyrosine kinases, autocrine or paracrine stimulation may contribute to the neoplastic state of the tumor cell. Some epithelial cancers overexpress one or more members of the receptor tyrosine kinase family. EGF receptors, IGF-I receptors and HER-2/neu are overexpressed in lung, bladder, breast, head and neck and ovarian cancers. Therefore, ligands for the tyrosine kinase receptors may be useful in the treatment of cancers.

The prototypical tyrosine phosphatase receptor is CD45. CD45 plays a crucial role in T cell activation through its removal of phosphate from a negative regulatory site on the src family kinase.

The serine/threonine kinase receptors recognize TGF, bone morphogenetic factors and other activins as ligands. Ligand binding leads to activation of the receptor kinase. Bone morphogenetic factors are important in bone formation and in determining ventral dorsal orientation in the developing embryo. TGF induces fibroblast proliferation but inhibits the proliferation of most cell types. Loss of expression or loss of function of TGF receptors occurs in several tumor types including colon cancer and lymphomas.

Guanylate cyclase receptors mediate the activity of atrial natriuretic peptides. They are made up of a large extracellular and intracellular domains (440-700 residues). Ligand binding leads to dimerization of the receptor. This association of the intracellular kinase domains leads to autophosphorylation. The phosphorylated sites provide high affinity binding sites for other intracellular proteins. The intracellular proteins have a highly conserved region of about 100

residues referred to as the SH2 domains. The proteins that contain SH2 domains contribute to the signaling cascades (e.g. activation of protein lipase C).

4.   Nuclear Steroid Receptors and Membrane-bound Steroid Receptors

Nuclear steroid receptors regulate DNA transcription, leading to the synthesis of specific proteins and the production of cellular effects. These are large monomeric proteins of 400 to 1000 residues incorporating a highly conserved region of 60 residues in the middle of the molecule. Glucocorticoids enhance the production of lipocortin accounting for the inflammatory properties of this receptor. Ligands for the receptor include steroid hormones such as estrogens, progestrins, and androgens, thyroid hormones, vitamin D and retinoids. For the glucocorticoid receptor, drugs that are activating ligands include prednisone, dexamethasone and betamethasone that provide therapy for the treatment of arthritis and carditis. For the mineralcorticoid receptor, desoxycorticosterone provides treatment for Addisons disease. For the androgen and estrogen receptor, anti-androgen and anti-estrogen ligands are relevant for the treatment of benign prostatic hyperplasia and breast carcinoma respectively. Selective estrogen receptor modulators (SERMs) include tamoxifen, raloxifene, and antoandrogens including cypoterone and flutamde.

Examples of ligands which bind to nuclear steroid receptors and their therapeutic indications include the following:

Receptor	Current and Potential Therapeutic Indication(s)	Drugs/Ligands
Corticosteroid	Allergic rhinitis, Urticaria, arthritis, Asthma, Dermatitis, Psoriasis, autoimmune disease, inflammation, IBD, COPD	mometasone, deflazacort, beclomethasone, budesonide, fluticasone, betamethasone, rimexolone, CBP-2011, CBP-2012, CBP-1011, AGN-191743, methylprednisolone aceponate, triamcinolone acetonide, Fiser A608, halobetasol propionate, ALX-25, rofleponide

Receptor	Current and Potential Therapeutic Indication(s)	Drugs/Ligands
PPAR γ	Type II Diabetes (NIDDM), atherosclerosis, hyperlipidemia	Rosiglitazone, troglitazone, pioglitazone, Englitazone, LG-101280, GW-2331, MCC-555, GW-2570, AD-5075, AHG-255, KRP-297, darglitazone, GI-262570, BRL-48482,
Retinoid X-α (RXR)	Acne, Keratosis, Psoriasis, Ichthyosis, Neoplasm, Restenosis, Prostatic hypertrophy, Systemic lupus erythematosus, Breast tumor, Leukemia, Lymphoma, Metabolic disorder, Carcinoma, Myeloproliferative disorder, Skin infection, Solid tumor, Squamous cell carcinoma, Allergy, Dermatological disease, Osteoporosis, Non-insulin dependent diabetes, Lung tumor, Genital system disease, Colon tumor, Myeloid Leukemia	Tazarotene, Alitretinoin, Bexarotene, ALRT-1550, ALRT-326/ALRT-4204, ALRT-268, LG-100264, ER-65250, LG-100754, LG-100264, MX-895
Estrogen	Osteoporosis, breast cancer, heart disease, depression, menopausal disorders, contraception	Tamoxifen, droloxifene, idoxifene, raloxifene, dihydroraloxifene, levormeloxifene, LY-353381, LY-355124, TAT-59, GW-5638, TSE-424, EM-652, CP-336156, ERA-923, LY-326315, LY-357489, ZM-189154, CCRL-1054

Cell surface steroid receptors can be distinguished from the nuclear
receptors (Harold H. Zakon[39] and Gianna Fiorelli et al.[40]). These cell surface
receptors are coupled to signal transduction pathways in the cell. Zakon[39]
describes how surface steroids can be coupled to second messenger systems and
control the activity of ion channels in this way. The presence of signaling cell
surface binding sites for estradiol has been reported in osteoblast- and
osteoclast-like cells. Two mechanisms of action have been reported for these
ligands: regulation of gene expression, and initiation of responses.

5.   Adhesion Molecules as Cell Surface Receptors

The adhesion of cells to molecules of the extracellular matrix or to other cells is a fundamental theme in biological processes that encompasses events that include signal transduction, regulation of the immune system, cell growth and wound healing. As a result there are a number of therapeutic applications for mediating/inhibiting these interactions, which includes the use of inhibitors of cell adhesion as anti-inflammatory agents. A number of classes of molecules have been implicated in these cell-cell and cell-matrix interactions. These include the following molecules: selectins (E, L and P), integrins ($\alpha_x\beta_y$), the IgG superfamily, fibrinogen and laminin.

Selectins are transmembrane glycoproteins of the vascular system that are involved in both lymphocyte and leukocyte adhesion and play a pivotal role in inflammation by allowing the attachment of leukocytes to endothelial cells. Integrins are a family of heterodimeric transmembrane adhesion molecules. Integrin $\alpha_2\beta_3$ is expressed on platelets and becomes exposed on thrombin activation. The IgG Superfamily includes VCAM, ICAM, and NCAM. Fibrinogen is a serum protein that is involved in the blood clotting cascade and binds to the platelet integrin $\alpha_2\beta_3$. The binding of fibrinogen to platelet integrin plays a key role in thrombosis. Fibronectin is a widely distributed glycoprotein present in most extracellular matrices. It's principle functions appear to be in cellular migration during development and wound healing, regulation of cell growth and differentiation and haemostasis/thrombosis. Laminins are a family of large glycoproteins that are distributed ubiquitously in basement membranes. This family of molecules have roles in development, differentiation and migration through their ability to interact with cells via cell-surface receptors, including the integrins (George A. Heavner[38]).

Examples of ligands which bind to adhesion receptors include the following:

Receptor	Current and Potential Therapeutic Indication(s)	Drugs/Ligands
Integrins		
gpIIb/IIIa	Unstable Angina, Myocardial Infarction, cerebrovascular ischemia, thromboembolism, peripheral vascular disease, restenosis	Sibrafiban, fradafiban, lotrafiban, lefradafiban, RWJ-50042, tirofiban, xemilofiban, XR-300, L-734217, L-738167, L-709780, L-703014, G-7464, G-7582, G-7584, G-7442, G-7570, DMP-728, XT-111, PS-028, CGH-400, PSA-0613, MS-180, NSL-95317, RPR-110173, Ro-43-8857, SA-202, TRM-147, TRM-153, XV454-7, TAK-029, EF-5077, ZD-2486, RWJ-53308, T-250, SM-20302, TS-943, SC-57345, S-1762
LFA-1	Rheumatoid arthritis, psoriasis	RWJ-50271, BIRT-377
Vitronectin (alpha-v beta-3)	restenosis, atherosclerosis, angiogenesis, cancer, osteoporosis, thromboembolism	SB-265123, SD-183, XT-199, V-0223, XT-199-4, V-0005, V-0519,V-0245,L-748415
VLA-4 (alpha-4-beta-1)	asthma, allergy, IBD, rheumatoid arhtritis, MS	BIO-1050, Bio-1211, Bio-1272, Bio-1515, TBC-3233, ZD7349
Selectins		
E-Selectin	Asthma, arthritis, rhinitis, reperfusion injury, inflammation, transplant rejection, atopic dermatitis, psoriasis, septic shock	TBC-1269, BMS-190394, CGP-69669A, GM-1925, GM-1380, GM-2296, GSC-150, GM-1986, GM-2941, PDN-26117
L-Selectin	Asthma, reperfusion injury, inflammation, transplant rejection, ARDS, septic shock	TBC-1269, BMS-190394, GSC-150, GM-1986, GM-2941, PDN-26117
P-Selectin	Arthritis, reperfusion injury, inflammation, ARDS	TBC-1269, BMS-190394, CGP-69669A, GM-1925, GM-1380, GM-2296, GSC-150, GM-1986, GM-2941, PDN-26117

In general terms a ligand may be an activator or an inhibitor. In the case of an activator, on binding with a receptor, the ligand will activate the functional response of the receptor. Alternatively, in the case of an inhibitor, a ligand will bind to the receptor but it does not activate the receptor. Instead, it may prevent the binding of an activating ligand.

Within the ligands that bind to G-protein cellular receptors, ligands may be specifically classified as follows:

1. Full agonists - ligands that when bound trigger the maximum activity seen by natural ligands

2. Partial agonists- ligands that when bound trigger sub-maximal activity

3. Antagonist- ligands that when bound inhibit or prevent the activity arising from a natural ligand binding to the receptor. Antagonists may be of the surmontable class (results in the parallel displacement of the dose-response curve of the agonist to the right in a dose dependent fashion without reducing the maximal response for the agonist) or insurmountable class (results in depression of the maximal response for a given agonist with or without the parallel shift).

4. Inverse antagonist-ligands that when bound decrease the basal activity of the unbound receptor (if any).

There are four fundamental properties that are measurable pertaining to the interaction of a ligand with its receptor.

1) The affinity of the ligand for the receptor, which relates to the energetics of the binding.

2) The efficacy of the ligand for the receptor, which relates to the functional downstream activity of the ligand.

3) The kinetics of the ligand for the receptor, which defines the onset of action and the duration of action.

4) The desensitization of the receptor for the ligand.

With regard to the ligand, it is the combination of these properties that provides the foundation for defining the nature of the functional response. Thus an activating ligand (or agonist) has affinity for the receptor and downstream efficacy. In contrast, an inhibiting ligand (antagonist) has affinity for the receptor but no efficacy.

Selectivity defines the ratios of affinities or the ratios of efficacies of a given ligand compared across two receptors. It is the selectivity of a specific drug that provides the required biological profile. For example, in certain therapeutic settings, it is currently thought that a highly selective drug may be preferred (eg.

5 Losartan (Cozaar), an antihypertensive, is a highly selective antagonist for the AT1 receptor). In contrast, it considered that a drug with a broad spectrum of receptor activity may be preferred in other therapeutic settings (eg. cisapride, for the treatment of gastric motility disorder and gastro-esophageal reflux disease, displays a breadth of activity as a $5HT_4$ agonist and $5HT_3$ antagonist).

10 The pharmaceuticals currently developed that bind to receptors have the clinical shortcomings of low efficacy, low affinity, poor safety profile, lack of selectivity or overselectivity for the intended receptor, and suboptimal duration of action and onset of action. Thus there continues to exist a need for new pharmaceuticals having improved therapeutic activities.

15 Accordingly, it would be beneficial to develop ligands that have improved affinity, efficacy, selectivity, onset of action and duration of action.

1. Affinity of ligand for target receptor

An increase in affinity may contribute to reducing the dose of ligand required to induce the desired therapeutic effect. A reduction in affinity will

20 remove activity and may contribute to the selectivity profile for a ligand.

2. Efficacy of ligand at a target receptor (functional effect)

An increased efficacy can lead to a reduction in the dose required to mediate the desired therapeutic effect. This increase in efficacy may arise from an improved positive functional response of the ligand or a change from a partial to

25 full agonist profile. Reduced efficacy of a full agonist to a partial agonist may provide clinical benefit by providing a moderated biological response e.g. Zolmitriptan is a partial agonist for the 5HT1b/d receptors.

3. Selectivity of ligand compared across receptor subtypes

An increase in the selectivity of the ligand requires that the affinity or efficacy of the ligand at other receptors is reduced relative to the desired receptor. For example ipratropium is a non-selective ligand showing activity at a number of muscarinic receptor subtypes resulting in undesirable side-effects.

5 A decrease in the selectivity of the ligand may also be desired. For example the angiotensin II endogenous ligand activates both the AT1 and AT2 receptor subtypes. However, Losartan is a selective AT1 receptor antagonist.

4. Onset of Action

More rapid onset of action is often preferred.

10 5. Duration of Action

An increased duration of action may be preferred. For example β_2 adrenergic agonists such as albuterol have a relatively short duration of action of approximately 3-4 hours. Migraineurs also suffer rebound headache after treatment with sumatriptan.

15 6. Desensitization of the receptor for the ligand.

Desensitization is best defined as the variety of processes by which the functional interaction of the receptor with its G-protein are influenced. These processes lead ultimately to a reduction in cellular response to the activating agonist. Such phenomena are most often observed during prolonged stimulation of

20 the receptor. The two main pathways for receptor desensitization are reduction in receptor density or changes in receptor structure by phosphorylation mechanisms.

Receptor density is altered by receptor sequestration. This is a reversible process that is observable within minutes and is a dynamic sorting of receptors with receptors being cycled to and from the membrane. On the other hand

25 receptor down regulation is generally slower, on the order of hours and is irreversible, involving destruction of the receptor. Finally, receptor density may be affected by an alteration in the rate of synthesis. The rate of β_2 mRNA synthesis and degradration are controlled by levels of c-AMP within the cell.

Alternatively receptor desensitization may occur through changes in receptor structure. The receptor may be phosphorylated. For example, agonist induced activation of the β_2-adrenergic receptor, which is positively coupled to adenylate cyclase through Gs, results in an elevation in the levels of c-AMP and an

5   increase in the activity of protein kinase A. This kinase can readily phosphorylate a consensus site in the third intracellular loop of the receptor. The phosphorylated β_2-adrenergic receptor exhibits significantly reduced coupling to Gs. Besides PKA, the G-protein coupled receptor kinases (GRK) are also involved in the desenitization of GPCRs. For the β_2-adrenergic receptor, there are two of these

10   kinases bARK1 and bARK2. These GRKs are more specific and will only phosphorylate an agonist activated receptor. Furthermore this GRK desensitization requires an arrestin protein.

Possible roles for multibinding ligands would be to mediate changes in the desensitzation mechanisms. It may also be useful to increase receptor

15   desensitization.

Receptor oligmerization also plays a role in receptor function. This is best exemplified in the area of growth receptors that are known to act functionally and structurally as dimers, e.g. EGF-R and interferon receptor. Importantly for the EGF-R receptor, it is proposed that it is this dimerization that provides the high

20   affinity binding sites. It is also known that dimerization is involved in the functioning of the steroid receptor. Preliminary evidence is beginning to appear on the importance of oligomerization in G-protein coupling and signalling. It is proposed that receptor oligomerization may play a role in different receptor functions such as mediating coupling of the G-protein or receptor internalization.

25   A functional role for receptor oligomerization has been proposed for the adrenergic receptor (Bouvier et al.[41] and Terence E. Hebert et al.[42]) and the opioid receptor (Cvejic et al.[43]). In addition, higher molecular weight species have been observed for a range of GPCRs that includes muscarinic receptors, 5HT receptors, dopamine receptors, m-GluR receptors, NK1 receptors and others. Receptor

dimerization has also been noted in the angiotensin AT1 receptor (Catherine Monnot et al.[44]).

Expression of dopaminergic D3 receptor dimers and tetramers in brain and in transfected cells has been obeserved (Esther A. Nimchinsky et al.[45]).

Accordingly, there is a need to develop ligands that are more specific with increased potency for the various receptors.

SUMMARY OF THE INVENTION

This invention is directed to novel multi-binding compounds that bind cellular receptors. The binding of these compounds to such cellular receptors can be used to treat pathologic conditions mediated by such cells.

Accordingly, in one of its composition aspects, this invention is directed to a multi-binding compound comprising 2 to 10 ligands which may be the same or different and which are covalently attached to a linker or linkers which may be the same of different, each of said ligands comprising a ligand domain capable of binding to a cellular receptor, with the following provisos:

(a)   the ligand does not bind to a 5-HT1b receptor, a 5-HT1d receptor, a 5-HT1f receptor, a β2-adrenergic receptor, a M2 muscarinic receptor, a M3 muscarinic receptor or an opioid receptor ;

(b)   when a first ligand is a peptide, then additional ligands do not bind to neurokinin 2 receptor or are not peptides;

(c)   when the multibinding compound comprises two ligands having a tetraazacrown capable of binding to a CCR5 or CXCR4 receptor, then the linker is not a polymethylene group;

(d)   when the multibinding compound comprises two ligands having a hexestrol moiety capable of binding to an estrogen receptor, then the linker is not a polymethylene group;

(e)   when the multibinding compound is capable of binding to an α-adrenergic receptor, then a ligand is not N,N'-(bis-(5-aminopentyl)cystamine (APC) or an analog thereof;

(f)   when a first ligand is 1-(aryloxy)-2-hydroxypropanolamine moiety and is capable of binding to a β- adrenergic receptor, then a linker is not a polymethylene or poly(ethyleneoxide) group;

(g)   when a first ligand is 2-allylphenyl, 4-(2-methoxyethyl)phenyl, 1-naphthyl, or 4-methoxyphenyl moiety and is capable of binding to the β1-adrenergic receptor, then the linker is not a Jeffamine;

(h)   when a first ligand is a sLeX moiety and is capable of binding to a selectin, then the linker is not a polymethylene or poly(ethyleneoxide) group;

(i)   when a first ligand is a mannose moiety and is capable of binding to a selectin, then the linker is not a poly(arylene) group;

(j)   when a first ligand is a 2-(3, 4-dihydroxybenzyl pyrrolidine) moiety and is capable of binding to a dopamine receptor and a linker is an alkylene group, then the second ligand is not a 2-(3, 4-dihydroxybenzyl pyrrolidine) or an N-ethyl (3, 4-dihydroxyphenethyl amine) moiety; and

(k)   when a first ligand is a 2-phenylbenzimidazole moiety and is capable of binding a dopamine receptor and a linker is an alkylene, alkenylene or arylalkylene group then a second ligand is not a 2-phenylbenzimidazole or a benzimidazole moiety.

The multi-binding compounds of this invention are preferably represented by formula I:

$$(L)_p(X)_q \hspace{4cm} I$$

wherein each L is independently selected from ligands comprising a ligand domain capable of binding to a cellular receptor; X is independently a linker; p is an integer of from 2 to 10; q is an integer of from 1 to 20, with the following provisos:

(a)   the ligand is not capable of binding to a 5-HT1b receptor, a 5-HT1d receptor, a 5-HT1f receptor, a beta-2-adrenergic receptor, a M2 muscarinic receptor, M3 muscarinic receptor or an opioid receptor;

(b)   when, in formula I, p is 2, q is 1 and the first L is a peptide, then the second L does not bind to neurokinin 2 receptor or is not a peptide;

(c)   when in formula I, X is a polymethylene group, p is 2 and q is 1, then L is not a tetraazacrown moiety capable of binding to a CCR5 or CXCR4 receptor;

(d)   when in formula I, X is a polymethylene group, p is 2 and q is 1, then L is not hexestrol moiety capable of binding to an estrogen receptor;

(e)   when in formula I, p is 2 and q is 1, then L is not an analog of N,N'-(bis-(5-aminopentyl)cystamine (APC) capable of binding to an α-adrenergic receptor;

(f)   when in formula I, X is a polymethylene or poly(ethyleneoxide) group, p is 2 and q is 1, then L is not a 1-(aryloxy)-2-hydroxypropanolamine moiety capable of binding to an β1-adrenergic receptor;

(g)   when in formula I, X is a Jeffamine, p is 2 and q is 1, then L is not a 2-allylphenyl, 4-(2-methoxyethyl)phenyl, 1-naphthyl, or 4-methoxyphenyl moiety capable of binding to an β1-adrenergic receptor;

(h)   when in formula I, X is a polymethylene or poly(ethyleneoxide) group, p is 2 and q is 1, then L is not a sLeX moiety capable of binding to a selectin;

(i)   when in formula I, X is a poly(arylene) group, p is 2 or 3 and q is 1, then L is not a mannose moiety capable of binding to a selectin;

(j)   when in formula I, X is an alkylene group, p is 2, q is 1 and the first L is a 2-(3, 4-dihydroxybenzyl pyrrolidine) moiety capable of binding to a dopamine receptor, then the second L is not a 2-(3, 4-dihydroxybenzyl pyrrolidine) or an N-ethyl (3, 4-dihydroxyphenethyl amine) moiety capable of binding to a dopamine receptor; and

(k)   when in formula I, X is an alkylene, alkenylene or arylalkylene group, p is 2, q is 1 and the first L is a 2-phenylbenzimidazole moiety capable of binding to a dopamine receptor, then the second L is not a 2-phenylbenzimidazole or a benzimidazole moiety capable of binding to a dopamine receptor. Preferably, q is less than p.

Preferably, binding of the multibinding compounds to the cell relates to cells which mediate mammalian or avian pathologic conditions and such binding modulates these conditions.

In another of its composition aspects, this invention is directed to a pharmaceutical composition comprising a pharmaceutically acceptable excipient and an effective amount of a multi-binding compound or a pharmaceutically acceptable salt thereof comprising 2 to 10 ligands which may be the same or different and which are covalently attached to a linker or linkers which may be the same or different, each of said ligands comprising a ligand domain capable of binding to one or more cellular receptors mediating mammalian pathologic conditions thereby modulating the pathologic condition with the following provisos:

(a)   the ligand does not bind to a 5-HT1b receptor, a 5-HT1d receptor, a 5-HT1f receptor, a β2-adrenergic receptor, a M2 muscarinic receptor, a M3 muscarinic receptor or an opioid receptor ;

(b)   when a first ligand is a peptide, then additional ligands do not bind to neurokinin 2 receptor or are not peptides;

(c)   when the multibinding compound comprises two ligands having a tetraazacrown capable of binding to a CCR5 or CXCR4 receptor, then the linker is not a polymethylene group;

(d)   when the multibinding compound comprises two ligands having a hexestrol moiety capable of binding to an estrogen receptor, then the linker is not a polymethylene group;

(e)   when the multibinding compound is capable of binding to an α-adrenergic receptor, then a ligand is not N,N'-(bis-(5-aminopentyl)cystamine (APC) or an analog thereof;

(f)   when a first ligand is 1-(aryloxy)-2-hydroxypropanolamine moiety and is capable of binding to an β- adrenergic receptor, then a linker is not a polymethylene or poly(ethyleneoxide) group;

(g)   when a first ligand is 2-allylphenyl, 4-(2-methoxyethyl)phenyl, 1-naphthyl, or 4-methoxyphenyl moiety and is capable of binding to the β1-adrenergic receptor, then the linker is not a Jeffamine;
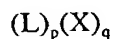
(h)   when a first ligand is a sLeX moiety and is capable of binding to a selectin, then the linker is not a polymethylene or poly(ethyleneoxide) group;

(i)   when a first ligand is a mannose moiety and is capable of binding to a selectin, then the linker is not a poly(arylene) group;

(j)   when a first ligand is a 2-(3, 4-dihydroxybenzyl pyrrolidine) moiety and is capable of binding to a dopamine receptor and a linker is an alkylene group, then the second ligand is not a 2-(3, 4-dihydroxybenzyl pyrrolidine) or an N-ethyl (3, 4-dihydroxyphenethyl amine) moiety; and

(k)   when a first ligand is a 2-phenylbenzimidazole moiety and is capable of binding a dopamine receptor and a linker is an alkylene, alkenylene or arylalkylene group then a second ligand is not a 2-phenylbenzimidazole or a benzimidazole moiety..

In still another of its composition aspects, this invention is directed to a pharmaceutical composition comprising a pharmaceutically acceptable excipient and an effective amount of a multi-binding compound represented by formula I:

$$(L)_p(X)_q \qquad\qquad \text{I}$$

wherein each L is independently selected from ligands comprising a ligand domain capable of binding to a cellular receptor mediating mammalian pathologic

conditions; X is a linker; p is an integer of from 2 to 10; q is an integer of from 1 to 20; with the following provisos:

(a) the ligand is not capable of binding to a 5-HT1b receptor, a 5-HT1d receptor, a 5-HT1f receptor, a beta-2-adrenergic receptor, a M2 muscarinic receptor, M3 muscarinic receptor or an opioid receptor;

(b) when, in formula I, p is 2, q is 1 and the first L is a peptide, then the second L does not bind to neurokinin 2 receptor or is not a peptide;

(c) when in formula I, X is a polymethylene group, p is 2 and q is 1, then L is not a tetraazacrown moiety capable of binding to a CCR5 or CXCR4 receptor;

(d) when in formula I, X is a polymethylene group, p is 2 and q is 1, then L is not hexestrol moiety capable of binding to an estrogen receptor;

(e) when in formula I, p is 2 and q is 1, then L is not an analog of N,N'-(bis-(5-aminopentyl)cystamine (APC) capable of binding to an α-adrenergic receptor;

(f) when in formula I, X is a polymethylene or poly(ethyleneoxide) group, p is 2 and q is 1, then L is not a 1-(aryloxy)-2-hydroxypropanolamine moiety capable of binding to an β1-adrenergic receptor;

(g) when in formula I, X is a Jeffamine, p is 2 and q is 1, then L is not a 2-allylphenyl, 4-(2-methoxyethyl)phenyl, 1-naphthyl, or 4-methoxyphenyl moiety capable of binding to an β1-adrenergic receptor;

(h) when in formula I, X is a polymethylene or poly(ethyleneoxide) group, p is 2 and q is 1, then L is not a sLeX moiety capable of binding to a selectin;

(i) when in formula I, X is a poly(arylene) group, p is 2 or 3 and q is 1, then L is not a mannose moiety capable of binding to a selectin;

(j) when in formula I, X is an alkylene group, p is 2, q is 1 and the first L is a 2-(3, 4-dihydroxybenzyl pyrrolidine) moiety capable of binding to a dopamine receptor, then the second L is not a 2-(3, 4-dihydroxybenzyl pyrrolidine)

or an N-ethyl (3, 4-dihydroxyphenethyl amine) moiety capable of binding to a dopamine receptor; and

(k) when in formula I, X is an alkylene, alkenylene or arylalkylene group, p is 2, q is 1 and the first L is a 2-phenylbenzimidazole moiety capable of binding to a dopamine receptor, then the second L is not a 2-phenylbenzimidazole or a benzimidazole moiety capable of binding to a dopamine receptor, and pharmaceutically acceptable salts thereof. Preferably, q is less than p.

In one of its method aspects, this invention is directed to a method for treating a mammalian or avian pathologic condition mediated by receptors which method comprises administering to said mammal or bird an effective amount of a pharmaceutical composition comprising a pharmaceutically acceptable excipient and a multi-binding compound or a pharmaceutically acceptable salt thereof comprising 2 to 10 ligands which may be the same or different and which are covalently attached to a linker or linkers which may be the same or different, each of said ligands comprising a ligand domain capable of binding to a cellular receptor mediating mammalian pathologic conditions with the following provisos:

(a) the ligand does not bind to a 5-HT1b receptor, a 5-HT1d receptor, a 5-HT1f receptor, a β2-adrenergic receptor, a M2 muscarinic receptor, a M3 muscarinic receptor or an opioid receptor ;

(b) when a first ligand is a peptide, then additional ligands do not bind to neurokinin 2 receptor or are not peptides;

(c) when the multibinding compound comprises two ligands having a tetraazacrown capable of binding to a CCR5 or CXCR4 receptor, then the linker is not a polymethylene group;

(d) when the multibinding compound comprises two ligands having a hexestrol moiety capable of binding to an estrogen receptor, then the linker is not a polymethylene group;

(e)   when the multibinding compound is capable of binding to an α-adrenergic receptor, then a ligand is not N,N'-(bis-(5-aminopentyl)cystamine (APC) or an analog thereof;

(f)   when a first ligand is 1-(aryloxy)-2-hydroxypropanolamine moiety and is capable of binding to an β- adrenergic receptor, then a linker is not a polymethylene or poly(ethyleneoxide) group;

(g)   when a first ligand is 2-allylphenyl, 4-(2-methoxyethyl)phenyl, 1-naphthyl, or 4-methoxyphenyl moiety and is capable of binding to the β1-adrenergic receptor, then the linker is not a Jeffamine;

(h)   when a first ligand is a sLeX moiety and is capable of binding to a selectin, then the linker is not a polymethylene or poly(ethyleneoxide) group;

(i)   when a first ligand is a mannose moiety and is capable of binding to a selectin, then the linker is not a poly(arylene) group;

(j)   when a first ligand is a 2-(3, 4-dihydroxybenzyl pyrrolidine) moiety and is capable of binding to a dopamine receptor and a linker is an alkylene group, then the second ligand is not a 2-(3, 4-dihydroxybenzyl pyrrolidine) or an N-ethyl (3, 4-dihydroxyphenethyl amine) moiety; and

(k)   when a first ligand is a 2-phenylbenzimidazole moiety and is capable of binding a dopamine receptor and a linker is an alkylene, alkenylene or arylalkylene group then a second ligand is not a 2-phenylbenzimidazole or a benzimidazole moiety.

In one of its method aspects, this invention is directed to a method for treating a mammalian or avian pathologic condition mediated by cellular receptors which method comprises administering to said mammal or bird an effective amount of a pharmaceutical composition comprising a pharmaceutically acceptable excipient and a multi-binding compound represented by formula I:

$$(L)_p(X)_q \qquad\qquad \text{I}$$

wherein each L is independently selected from ligands comprising a ligand domain capable of binding to a cellular receptor mediating mammalian pathologic conditions; X is a linker; p is an integer of from 2 to 10; q is an integer of from 1 to 20, with the following provisos:

     (a)   the ligand is not capable of binding to a 5-HT1b receptor, a 5-HT1d receptor, a 5-HT1f receptor, a beta-2-adrenergic receptor, a M2 muscarinic receptor, M3 muscarinic receptor or an opioid receptor;

     (b)   when, in formula I, p is 2, q is 1 and the first L is a peptide, then the second L does not bind to neurokinin 2 receptor or is not a peptide;

     (c)   when in formula I, X is a polymethylene group, p is 2 and q is 1, then L is not a tetraazacrown moiety capable of binding to a CCR5 or CXCR4 receptor;

     (d)   when in formula I, X is a polymethylene group, p is 2 and q is 1, then L is not hexestrol moiety capable of binding to an estrogen receptor;

     (e)   when in formula I, p is 2 and q is 1, then L is not an analog of N,N'-(bis-(5-aminopentyl)cystamine (APC) capable of binding to an α-adrenergic receptor;

     (f)   when in formula I, X is a polymethylene or poly(ethyleneoxide) group, p is 2 and q is 1, then L is not a 1-(aryloxy)-2-hydroxypropanolamine moiety capable of binding to an β1-adrenergic receptor;

     (g)   when in formula I, X is a Jeffamine, p is 2 and q is 1, then L is not a 2-allylphenyl, 4-(2-methoxyethyl)phenyl, 1-naphthyl, or 4-methoxyphenyl moiety capable of binding to an β1-adrenergic receptor;

     (h)   when in formula I, X is a polymethylene or poly(ethyleneoxide) group, p is 2 and q is 1, then L is not a sLeX moiety capable of binding to a selectin;

     (i)   when in formula I, X is a poly(arylene) group, p is 2 or 3 and q is 1, then L is not a mannose moiety capable of binding to a selectin;

(j)   when in formula I, X is an alkylene group, p is 2, q is 1 and the

first L is a 2-(3, 4-dihydroxybenzyl pyrrolidine) moiety capable of binding to a

dopamine receptor, then the second L is not a 2-(3, 4-dihydroxybenzyl pyrrolidine)

or an N-ethyl (3, 4-dihydroxyphenethyl amine) moiety capable of binding to a

5   dopamine receptor; and

(k)   when in formula I, X is an alkylene, alkenylene or arylalkylene

group, p is 2, q is 1 and the first L is a 2-phenylbenzimidazole moiety capable of

binding to a dopamine receptor, then the second L is not a 2-phenylbenzimidazole

or a benzimidazole moiety capable of binding to a dopamine receptor, and

10   pharmaceutically acceptable salts thereof. Preferably, q is less than p.

This invention is also directed to general synthetic methods for generating

large libraries of diverse multimeric compounds which multimeric compounds bind

cellular receptors and are candidates for possessing multibinding properties. The

diverse multimeric compound libraries provided by this invention are synthesized

15   by combining a linker or linkers with a ligand or ligands to provide for a library of

multimeric compounds wherein the linker and ligand each have complementary

functional groups permitting covalent linkage. The library of linkers is preferably

selected to have diverse properties such as valency, linker length, linker geometry

and rigidity, hydrophilicity or hydrophobicity, amphiphilicity, acidity, basicity,

20   polarizability and polarization. The library of ligands is preferably selected to

have diverse attachment points on the same ligand, different functional groups at

the same site of otherwise the same ligand, and the like.

This invention is also directed to libraries of diverse multimeric compounds

which multimeric compounds bind cellular receptors and are candidates for

25   possessing multibinding properties. These libraries are prepared via the methods

described above and permit the rapid and efficient evaluation of what molecular

constraints impart multibinding properties to a ligand or a class of ligands targeting

a receptor.

Accordingly, in one of its method aspects, this invention is directed to a method for identifying multimeric ligand compounds which bind cellular receptors possessing multibinding properties which method comprises:

(a)   identifying a ligand or a mixture of ligands wherein each ligand binds a cellular receptor and contains at least one reactive functionality;

(b)   identifying a library of linkers wherein each linker in said library comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand;

(c)   preparing a multimeric ligand compound library by combining at least two stoichiometric equivalents of the ligand or mixture of ligands identified in (a) with the library of linkers identified in (b) under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands; and

(d)   assaying the multimeric ligand compounds produced in (c) above to identify multimeric ligand compounds possessing multibinding properties.

In another of its method aspects, this invention is directed to a method for identifying multimeric ligand compounds which bind cellular receptors possessing multibinding properties which method comprises:

(a)   identifying a library of ligands wherein each ligand binds a cellular receptor and contains at least one reactive functionality;

(b)   identifying a linker or mixture of linkers wherein each linker comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand;

(c)   preparing a multimeric ligand compound library by combining at least two stoichiometric equivalents of the library of ligands identified in (a) with the linker or mixture of linkers identified in (b) under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands; and

(d)   assaying the multimeric ligand compounds produced in (c) above to identify multimeric ligand compounds possessing multibinding properties.

The preparation of the multimeric ligand compound library is achieved by either the sequential or concurrent combination of the two or more stoichiometric equivalents of the ligands identified in (a) with the linkers identified in (b). Sequential addition is preferred when a mixture of different ligands is employed to ensure heterodimeric or multimeric compounds are prepared. Concurrent addition of the ligands occurs when at least a portion of the multimer compounds prepared are homomultimeric compounds.

The assay protocols recited in (d) can be conducted on the multimeric ligand compound library or portions thereof produced in (c) above, or preferably, each member of the library is isolated by preparative liquid chromatography mass spectrometry (LCMS).

In one of its composition aspects, this invention is directed to a library of multimeric ligand compounds which may bind a cellular receptor and may possess multivalent properties which library is prepared by the method comprising:

(a)   identifying a ligand or a mixture of ligands which bind a cellular receptor wherein each ligand contains at least one reactive functionality;

(b)   identifying a library of linkers wherein each linker in said library comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand; and

(c)   preparing a multimeric ligand compound library by combining at least two stoichiometric equivalents of the ligand or mixture of ligands identified in (a) with the library of linkers identified in (b) under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands.

In another of its composition aspects, this invention is directed to a library of multimeric ligand compounds which may bind a cellular receptor and may possess multivalent properties which library is prepared by the method comprising:

(a)   identifying a library of ligands wherein each ligand binds a cellular receptor and contains at least one reactive functionality;

(b)   identifying a linker or mixture of linkers wherein each linker comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand; and

(c)   preparing a multimeric ligand compound library by combining at least two stoichiometric equivalents of the library of ligands identified in (a) with the linker or mixture of linkers identified in (b) under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands.

In a preferred embodiment, the library of linkers employed in either the methods or the library aspects of this invention is selected from the group comprising flexible linkers, rigid linkers, hydrophobic linkers, hydrophilic linkers, linkers of different geometry, acidic linkers, basic linkers, linkers of different polarization and/or polarizability and amphiphilic linkers. For example, in one embodiment, each of the linkers in the linker library may comprise linkers of different chain length and/or having different complementary reactive groups. Such linker lengths can preferably range from about 2 to 100Å.

In another preferred embodiment, the ligand or mixture of ligands is selected to have reactive functionality at different sites on said ligands in order to provide for a range of orientations of said ligand on said multimeric ligand compounds. Such reactive functionality includes, by way of example, carboxylic acids, carboxylic acid halides, carboxyl esters, amines, halides, pseudohalides, isocyanates, vinyl unsaturation, ketones, aldehydes, thiols, alcohols, anhydrides, boronates and precursors thereof. It is understood, of course, that the reactive functionality on the ligand is selected to be complementary to at least one of the reactive groups on the linker so that a covalent linkage can be formed between the linker and the ligand.

In other embodiments, the multimeric ligand compound is homomeric (i.e., each of the ligands is the same, although it may be attached at different points) or heteromeric (i.e., at least one of the ligands is different from the other ligands).

In addition to the combinatorial methods described herein, this invention provides for an iterative process for rationally evaluating what molecular constraints impart multibinding properties to a class of multimeric compounds or ligands targeting a receptor. Specifically, this method aspect is directed to a method for identifying multimeric ligand compounds possessing multibinding properties which method comprises:

(a)   preparing a first collection or iteration of multimeric compounds which is prepared by contacting at least two stoichiometric equivalents of the ligand or mixture of ligands which target a cellular receptor with a linker or mixture of linkers wherein said ligand or mixture of ligands comprises at least one reactive functionality and said linker or mixture of linkers comprises at least two functional groups having complementary reactivity to at least one of the reactive functional groups of the ligand wherein said contacting is conducted under conditions wherein the complementary functional groups react to form a covalent linkage between said linker and at least two of said ligands;

(b)   assaying said first collection or iteration of multimeric compounds to assess which if any of said multimeric compounds possess multibinding properties;

(c)   repeating the process of (a) and (b) above until at least one multimeric compound is found to possess multibinding properties;

(d)   evaluating what molecular constraints imparted multibinding properties to the multimeric compound or compounds found in the first iteration recited in (a)- (c) above;

(e)   creating a second collection or iteration of multimeric compounds which elaborates upon the particular molecular constraints imparting multibinding properties to the multimeric compound or compounds found in said first iteration;

(f)   evaluating what molecular constraints imparted enhanced multibinding properties to the multimeric compound or compounds found in the second collection or iteration recited in (e) above;

(g)   optionally repeating steps (e) and (f) to further elaborate upon said molecular constraints.

Preferably, steps (e) and (f) are repeated at least two times, more preferably at from 2-50 times, even more preferably from 3 to 50 times, and still more preferably at least 5-50 times.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1-11 illustrate numerous reaction schemes suitable for preparing linkers and, hence, multi-binding compounds of this invention.

Figure 12 illustrates examples of multi-binding compounds comprising 2 ligands attached in different formats to a linker.

Figure 13 illustrates examples of multi-binding compounds comprising 3 ligands attached in different formats to a linker.

Figure. 14 illustrates examples of multi-binding compounds comprising 4 ligands attached in different formats to a linker.

Figure 15 illustrates examples of multi-binding compounds comprising >4 ligands attached in different formats to a linker.

Figure 16 illustrates triptan C3 and C5 substituents.

Figure 17 illustrates several Sumatriptan building blocks.

Figure 18 illustrates several sites for multimerization of Sumatriptan.

Figures 19 - 23 illustrate several multimeric Sumatriptan compounds and convenient methods for their synthesis by the methods of this invention.

Figure 24 illustrates various muscarinic compounds.

Figure 25 illustrates the preferred sites for dimerization of the muscarinic family of compounds.

Figure 26 illustrates the different points for attachment of the linker on ipratropium and the resulting dimers.

Figures 27 and 28 illustrate the different linkers useful in making multimers for ipratropium.

Figure 29 illustrates various ipratropium dimers.

Figure 30 illustrates the synthesis of ipratropium dimers via quaternization.

Figure 31 illustrates the synthesis of ipratropium dimers via reductive amination.

Figure 32 illustrates the synthesis of ipratropium dimers via etherification.

Figure 33 illustrates the synthesis of ipratropium dimers via conjugate addition.

Figure 34 illustrates the two AT1 antagonists losartan and valsartan.

Figure 35 illustrates the AT1 antagonists Irbesartan, Candesartan, Eprosartan and Tasosartan.

Figure 36 illustrates the AT1 antagonists Telmisartan and Ripisartan.

Figure 37 illustrates the sites (M) that may be used to generate multi-binding compounds from Losartan.

Figures 38 and 39 illustrate the different points of attachment to Losartan.

Figure 40 illustrates the differing valencies of the multi-bonding compounds. Figure 41 illustrates the different framework cores.

Figure 42 illustrates the different orientations of binding elements within the multi-binding compounds.

Figure 43 illustrates the heterovalomers possible from AT1 antagonists.

Figure 44 illustrates the synthesis of 1-hydroxyl-linked Losartan multi-binding compound.

Figure 45 illustrates the synthesis of 2-hydroxyl-linked Losartan multi-binding compound.

Figure 46 illustrates the synthesis of tetrazole-linked Losartan multi-binding compound.

Figure 47 illustrates various β_2-adrenergic compounds.

Figure 48 illustrates preferred sites for attachment of linkers to albuterol. The M indicates the preferred attachment site.

Figure 49 illustrates various albuterol dimers.

Figure 50 illustrates different sites of attachment of the linker to albuterol and the resulting dimers.

Figures 51 and 52 illustrate the different linkers which may be used to generate multimers of albuterol.

Figures 53 and 54 exemplify different albuterol, albuterol/formeterol and albuterol/clenbuterol multimers.

Figures 55, 56, and 57 illustrate the different methods for the synthesis of bivalent analogs of salmeterol.

Figure 58 illustrates a method for the synthesis of a bivalent analog of alprenolol.

DETAILED DESCRIPTION OF THE INVENTION

Ligand (drug) interactions with cellular receptors are controlled by molecular interaction/recognition between the ligand and the receptor. In turn, such interaction can result in modulation or disruption of the biological processes/functions of these receptors and, in some cases, leads to cell death. Accordingly, when cellular receptors mediate mammalian pathologic conditions, interactions of ligands with the cellular receptor can be used to treat these conditions.

Without being limited, it is thought that a number of cellular receptors are amenable to interaction with a multi-binding compound of the present invention for the following reasons:

1)   Receptors with multiple binding sites within single receptor molecules:

a)   Some receptors have multiple sites for a single ligand, e.g. angiotensin;

b)   Some receptors have an accessory "weak" binding site in addition to the main ligand site, e.g. 5HT

5   c)   Some receptor-endogenous peptide ligands have different binding domains to the small molecule non-peptide antagonists. For example, the AT1 receptor binds two copies of the endogenous peptide agonist angiotensin II and the small molecule antagonist losartan; and

d)   Some receptors display allosteric binding sites for small molecules.
10   For example, the muscarinic family of receptors have been demonstrated to have allosteric binding sites

2)   Receptors that form oligomeric complexes.

Receptors that are known to form oligomeric species include the β_2 adrenergic; M3 receptors; tyrosine kinase and guanylate cyclase linked receptors;
15   insulin growth factors; nuclear receptors and many cytokine receptors. These receptors must dimerize to initiate their functional activity. Ligand binding leads to dimerization of the receptor. This association of the intracellular kinase domains leads to autophosphorylation. These phosphorylated sites provide high affinity binding sites for other intracellular proteins which contribute to cellular
20   signaling pathways. Also ligand-gated ion channel receptors form pentameric structures having multiple ligand-binding sites.

3)   Receptors that are closely spaced.

Although not interacting, some receptors are placed on a cell membrane at a distance that allows more than one receptor to be spanned with a multi-binding
25   ligand, e.g. synaptic receptors.

The interaction of a cellular receptor and a ligand may be described in terms of "affinity" and "specificity". The affinity and specificity of any given ligand/cellular receptor interaction are dependent upon the complementarity of

molecular binding surfaces and the energetic costs of complexation. Affinity is sometimes quantified by the equilibrium constant of complex formation.

Specificity relates to the difference in affinity between the same ligand binding to different ligand binding sites on the cellular receptor.

The multibinding compounds of this invention are capable of acting as multibinding agents and the surprising activity of these compounds arises at least in part from their ability to bind in a multivalent manner with one or more cellular receptors. Multivalent binding interactions are characterized by the concurrent interaction of multiple ligands with multiple ligand binding sites on one or more cellular receptors. Multivalent interactions differ from collections of individual monovalent interactions by imparting enhanced biological and/or therapeutic effect. Examples of multivalent binding interactions (e.g., trivalent) relative to monovalent binding interactions are shown below:
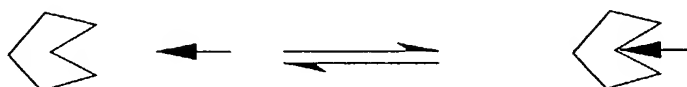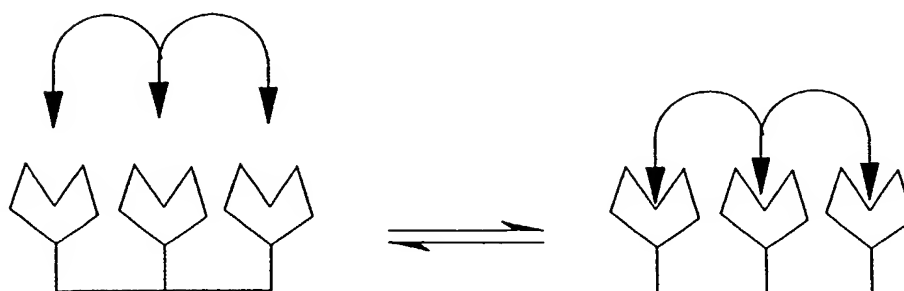
Just as multivalent binding can amplify binding affinities, it can also amplify differences in binding affinities, resulting in enhanced binding specificity as well as affinity.

Definitions:

Prior to discussing this invention in further detail, the following terms will first be defined.

The term "alkyl" refers to a monoradical branched or unbranched saturated hydrocarbon chain preferably having from 1 to 40 carbon atoms, more preferably 1 to 10 carbon atoms, and even more preferably 1 to 6 carbon atoms. This term is exemplified by groups such as methyl, ethyl, n-propyl, iso-propyl, n-butyl, iso-butyl, n-hexyl, n-decyl, tetradecyl, and the like.

The term "substituted alkyl" refers to an alkyl group as defined above, having from 1 to 5 substituents, and preferably 1 to 3 substituents, selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocyclooxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO$_2$-alkyl, -SO$_2$-substituted alkyl, -SO$_2$-aryl and -SO$_2$-heteroaryl.

The term "alkylene" refers to a diradical of a branched or unbranched saturated hydrocarbon chain, preferably having from 1 to 40 carbon atoms, more preferably 1 to 10 carbon atoms and even more preferably 1 to 6 carbon atoms. This term is exemplified by groups such as methylene (-CH$_2$-), ethylene (-CH$_2$CH$_2$-), the propylene isomers (e.g., -CH$_2$CH$_2$CH$_2$- and -CH(CH$_3$)CH$_2$-) and the like.

The term "substituted alkylene" refers to an alkylene group, as defined above, having from 1 to 5 substituents, and preferably 1 to 3 substituents, selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy,

amino, substituted amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocyclooxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -

SO-heteroaryl, -SO$_2$-alkyl, -SO$_2$-substituted alkyl, -SO$_2$-aryl and -SO$_2$-heteroaryl. Additionally, such substituted alkylene groups include those where 2 substituents on the alkylene group are fused to form one or more cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, heterocyclic or heteroaryl groups fused to the alkylene group. Preferably such fused groups contain from 1

to 3 fused ring structures.

The term "alkaryl" refers to the groups -alkylene-aryl and -substituted alkylene-aryl where alkylene, substituted alkylene and aryl are defined herein. Such alkaryl groups are exemplified by benzyl, phenethyl and the like.

The term "alkoxy" refers to the groups alkyl-O-, alkenyl-O-, cycloalkyl-O-, cycloalkenyl-O-, and alkynyl-O-, where alkyl, alkenyl, cycloalkyl, cycloalkenyl, and alkynyl are as defined herein. Preferred alkoxy groups are alkyl-O- and include, by way of example, methoxy, ethoxy, *n*-propoxy, *iso*-propoxy, *n*-butoxy, *tert*-butoxy, *sec*-butoxy, *n*-pentoxy, *n*-hexoxy, 1,2-dimethylbutoxy, and the like.

The term "substituted alkoxy" refers to the groups substituted alkyl-O-, substituted alkenyl-O-, substituted cycloalkyl-O-, substituted cycloalkenyl-O-, and substituted alkynyl-O- where substituted alkyl, substituted alkenyl, substituted cycloalkyl, substituted cycloalkenyl and substituted alkynyl are as defined herein.

The term "alkylalkoxy" refers to the groups -alkylene-O-alkyl,

alkylene-O-substituted alkyl, substituted alkylene-O-alkyl and substituted alkylene-O-substituted alkyl wherein alkyl, substituted alkyl, alkylene and substituted alkylene are as defined herein. Preferred alkylalkoxy groups are alkylene-O-alkyl and include, by way of example, methylenemethoxy ($-CH_2OCH_3$),

5   ethylenemethoxy ($-CH_2CH_2OCH_3$), n-propylene-iso-propoxy ($-CH_2CH_2CH_2OCH(CH_3)_2$), methylene-t-butoxy ($-CH_2-O-C(CH_3)_3$) and the like.

The term "alkylthioalkoxy" refers to the group -alkylene-S-alkyl, alkylene-S-substituted alkyl, substituted alkylene-S-alkyl and substituted alkylene-S-substituted alkyl wherein alkyl, substituted alkyl, alkylene and substituted

10   alkylene are as defined herein. Preferred alkylthioalkoxy groups are alkylene-S-alkyl and include, by way of example, methylenethiomethoxy ($-CH_2SCH_3$), ethylenethiomethoxy ($-CH_2CH_2SCH_3$), n-propylene-iso-thiopropoxy ($-CH_2CH_2CH_2SCH(CH_3)_2$), methylene-t-thiobutoxy ($-CH_2SC(CH_3)_3$) and the like.

The term "alkenyl" refers to a monoradical of a branched or unbranched

15   unsaturated hydrocarbon group preferably having from 2 to 40 carbon atoms, more preferably 2 to 10 carbon atoms and even more preferably 2 to 6 carbon atoms and having at least 1 and preferably from 1-6 sites of vinyl unsaturation. Preferred alkenyl groups include ethenyl ($-CH=CH_2$), n-propenyl ($-CH_2CH=CH_2$), iso-propenyl ($-C(CH_3)=CH_2$), and the like.

20   The term "substituted alkenyl" refers to an alkenyl group as defined above having from 1 to 5 substituents, and preferably 1 to 3 substituents, selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano,

25   halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocyclooxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, $-SO_2$-alkyl, $-SO_2$-substituted alkyl, $-SO_2$-aryl and $-SO_2$-heteroaryl.

The term "alkenylene" refers to a diradical of a branched or unbranched unsaturated hydrocarbon group preferably having from 2 to 40 carbon atoms, more preferably 2 to 10 carbon atoms and even more preferably 2 to 6 carbon atoms and having at least 1 and preferably from 1-6 sites of vinyl unsaturation. This term is

5 exemplified by groups such as ethenylene (-CH=CH-), the propenylene isomers (e.g., -CH$_2$CH=CH- and -C(CH$_3$)=CH-) and the like.

The term "substituted alkenylene" refers to an alkenylene group as defined above having from 1 to 5 substituents, and preferably from 1 to 3 substituents, selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl,

10 substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocyclooxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy,

15 hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, - SO-heteroaryl, -SO$_2$-alkyl, -SO$_2$-substituted alkyl, -SO$_2$-aryl and -SO$_2$-heteroaryl. Additionally, such substituted alkenylene groups include those where 2 substituents on the alkenylene group are fused to form one or more cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, heterocyclic or heteroaryl

20 groups fused to the alkenylene group.

The term "alkynyl" refers to a monoradical of an unsaturated hydrocarbon preferably having from 2 to 40 carbon atoms, more preferably 2 to 20 carbon atoms and even more preferably 2 to 6 carbon atoms and having at least 1 and preferably from 1-6 sites of acetylene (triple bond) unsaturation. Preferred alkynyl

25 groups include ethynyl (-C≡CH$_2$), propargyl (-CH$_2$C≡CH) and the like.

The term "substituted alkynyl" refers to an alkynyl group as defined above having from 1 to 5 substituents, and preferably 1 to 3 substituents, selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy,

amino, substituted amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocyclooxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, - SO-heteroaryl, -SO$_2$-alkyl, -SO$_2$-substituted alkyl, -SO$_2$-aryl and -SO$_2$-heteroaryl.

The term "alkynylene" refers to a diradical of an unsaturated hydrocarbon preferably having from 2 to 40 carbon atoms, more preferably 2 to 10 carbon atoms and even more preferably 2 to 6 carbon atoms and having at least 1 and preferably from 1-6 sites of acetylene (triple bond) unsaturation. Preferred alkynylene groups include ethynylene (-C≡C-), propargylene (-CHC≡C-) and the like.

The term "substituted alkynylene" refers to an alkynylene group as defined above having from 1 to 5 substituents, and preferably 1 to 3 substituents, selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocyclooxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, - SO-heteroaryl, -SO$_2$-alkyl, -SO$_2$-substituted alkyl, -SO$_2$-aryl and -SO$_2$-heteroaryl

The term "acyl" refers to the groups HC(O)-, alkyl-C(O)-, substituted alkyl-C(O)-, cycloalkyl-C(O)-, substituted cycloalkyl-C(O)-, cycloalkenyl-C(O)-, substituted cycloalkenyl-C(O)-, aryl-C(O)-, heteroaryl-C(O)- and heterocyclic- C(O)- where alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, heteroaryl and heterocyclic are as defined herein.

heteroaryloxy, heterocyclic, heterocyclooxy, aminoacyloxy, oxyacylamino, thioalkoxy, substituted thioalkoxy, thioaryloxy, thioheteroaryloxy, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO$_2$-alkyl, -SO$_2$-substituted alkyl, -SO$_2$-aryl, -SO$_2$-heteroaryl and trihalomethyl. Preferred aryl substituents include

5   alkyl, alkoxy, halo, cyano, nitro, trihalomethyl, and thioalkoxy.

The term "aryloxy" refers to the group aryl-O- wherein the aryl group is as defined above including optionally substituted aryl groups as also defined above.

The term "arylene" refers to the diradical derived from aryl (including substituted aryl) as defined above and is exemplified by 1,2-phenylene, 1,3-

10   phenylene, 1,4-phenylene, 1,2-naphthylene and the like.

The term "amino" refers to the group -NH$_2$.

The term "substituted amino refers to the group -NRR where each R is independently selected from the group consisting of hydrogen, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, alkenyl, substituted alkenyl,

15   cycloalkenyl, substituted cycloalkenyl, alkynyl, substituted alkynyl, aryl, heteroaryl and heterocyclic provided that both R's are not hydrogen.

The term "carboxyalkyl" refers to the groups "-C(O)O-alkyl", "-C(O)O-substituted alkyl", "-C(O)O-cycloalkyl", "-C(O)O-substituted cycloalkyl", "-C(O)O-alkenyl", "-C(O)O-substituted alkenyl", "-C(O)O-alkynyl" and "-C(O)O-

20   substituted alkynyl" where alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, alkenyl, substituted alkenyl, alkynyl and substituted alkynyl alkynyl are as defined herein.

The term "cycloalkyl" refers to cyclic alkyl groups of from 3 to 20 carbon atoms having a single cyclic ring or multiple condensed rings. Such cycloalkyl

25   groups include, by way of example, single ring structures such as cyclopropyl, cyclobutyl, cyclopentyl, cyclooctyl, and the like, or multiple ring structures such as adamantanyl, and the like.

The term "substituted cycloalkyl" refers to cycloalkyl groups having from

The term "acylamino" or "aminocarbonyl" refers to the group -C(O)NRR where each R is independently hydrogen, alkyl, substituted alkyl, aryl, heteroaryl, heterocyclic or where both R groups are joined to form a heterocyclic group (e.g., morpholino) wherein alkyl, substituted alkyl, aryl, heteroaryl and heterocyclic are as defined herein.

The term "aminoacyl" refers to the group -NRC(O)R where each R is independently hydrogen, alkyl, substituted alkyl, aryl, heteroaryl, or heterocyclic wherein alkyl, substituted alkyl, aryl, heteroaryl and heterocyclic are as defined herein.

The term "aminoacyloxy" or "alkoxycarbonylamino" refers to the group -NRC(O)OR where each R is independently hydrogen, alkyl, substituted alkyl, aryl, heteroaryl, or heterocyclic wherein alkyl, substituted alkyl, aryl, heteroaryl and heterocyclic are as defined herein.

The term "acyloxy" refers to the groups alkyl-C(O)O-, substituted alkyl-C(O)O-, cycloalkyl-C(O)O-, substituted cycloalkyl-C(O)O-, aryl-C(O)O-, heteroaryl-C(O)O-, and heterocyclic-C(O)O- wherein alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, aryl, heteroaryl, and heterocyclic are as defined herein.

The term "aryl" refers to an unsaturated aromatic carbocyclic group of from 6 to 20 carbon atoms having a single ring (e.g., phenyl) or multiple condensed (fused) rings (e.g., naphthyl or anthryl). Preferred aryls include phenyl, naphthyl and the like.

Unless otherwise constrained by the definition for the aryl substituent, such aryl groups can optionally be substituted with from 1 to 5 substituents, preferably 1 to 3 substituents, selected from the group consisting of acyloxy, hydroxy, thiol, acyl, alkyl, alkoxy, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, substituted alkyl, substituted alkoxy, substituted alkenyl, substituted alkynyl, substituted cycloalkyl, substituted cycloalkenyl, amino, substituted amino, aminoacyl, acylamino, alkaryl, aryl, aryloxy, azido, carboxyl, carboxylalkyl, cyano, halo, nitro, heteroaryl,

1 to 5 substituents, and preferably 1 to 3 substituents, selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano,

5   halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocyclooxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, - SO-heteroaryl,

10   -SO_2-alkyl, -SO_2-substituted alkyl, -SO_2-aryl and -SO_2-heteroaryl.

The term "cycloalkenyl" refers to cyclic alkenyl groups of from 4 to 20 carbon atoms having a single cyclic ring and at least one point of internal unsaturation. Examples of suitable cycloalkenyl groups include, for instance, cyclobut-2-enyl, cyclopent-3-enyl, cyclooct-3-enyl and the like.

15   The term "substituted cycloalkenyl" refers to cycloalkenyl groups having from 1 to 5 substituents, and preferably 1 to 3 substituents, selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano,

20   halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocyclooxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, - SO-heteroaryl,

25   -SO_2-alkyl, -SO_2-substituted alkyl, -SO_2-aryl and -SO_2-heteroaryl.

The term "halo" or "halogen" refers to fluoro, chloro, bromo and iodo.

The term "heteroaryl" refers to an aromatic group of from 1 to 15 carbon atoms and 1 to 4 heteroatoms selected from oxygen, nitrogen and sulfur within at least one ring (if there is more than one ring).

Unless otherwise constrained by the definition for the heteroaryl substituent, such heteroaryl groups can be optionally substituted with 1 to 5 substituents, preferably 1 to 3 substituents, selected from the group consisting of acyloxy, hydroxy, thiol, acyl, alkyl, alkoxy, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, substituted alkyl, substituted alkoxy, substituted alkenyl, substituted alkynyl, substituted cycloalkyl, substituted cycloalkenyl, amino, substituted amino, aminoacyl, acylamino, alkaryl, aryl, aryloxy, azido, carboxyl, carboxylalkyl, cyano, halo, nitro, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy, aminoacyloxy, oxyacylamino, thioalkoxy, substituted thioalkoxy, thioaryloxy, thioheteroaryloxy, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO$_2$-alkyl, -SO$_2$-substituted alkyl, -SO$_2$-aryl, -SO$_2$-heteroaryl and trihalomethyl. Preferred aryl substituents include alkyl, alkoxy, halo, cyano, nitro, trihalomethyl, and thioalkoxy. Such heteroaryl groups can have a single ring (e.g., pyridyl or furyl) or multiple condensed rings (e.g., indolizinyl or benzothienyl). Preferred heteroaryls include pyridyl, pyrrolyl and furyl.

The term "heteroaryloxy" refers to the group heteroaryl-O-.

The term "heteroarylene" refers to the diradical group derived from heteroaryl (including substituted heteroaryl), as defined above, and is exemplified by the groups 2,6-pyridylene, 2,4-pyridiylene, 1,2-quinolinylene, 1,8-quinolinylene, 1,4-benzofuranylene, 2,5-pyridnylene, 2,5-indolenyl and the like.

The term "heterocycle" or "heterocyclic" refers to a monoradical saturated unsaturated group having a single ring or multiple condensed rings, from 1 to 40 carbon atoms and from 1 to 10 hetero atoms, preferably 1 to 4 heteroatoms, selected from nitrogen, sulfur, phosphorus, and/or oxygen within the ring.

Unless otherwise constrained by the definition for the heterocyclic substituent, such heterocyclic groups can be optionally substituted with 1 to 5, and preferably 1 to 3 substituents, selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl,

aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocyclooxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl,

5 -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, $-SO_2$-alkyl, $-SO_2$-substituted alkyl, $-SO_2$-aryl and $-SO_2$-heteroaryl. Such heterocyclic groups can have a single ring or multiple condensed rings. Preferred heterocyclics include morpholino, piperidinyl, and the like.

Examples of nitrogen heterocycles and heteroaryls include, but are not

10 limited to, pyrrole, imidazole, pyrazole, pyridine, pyrazine, pyrimidine, pyridazine, indolizine, isoindole, indole, indazole, purine, quinolizine, isoquinoline, quinoline, phthalazine, naphthylpyridine, quinoxaline, quinazoline, cinnoline, pteridine, carbazole, carboline, phenanthridine, acridine, phenanthroline, isothiazole, phenazine, isoxazole, phenoxazine, phenothiazine,

15 imidazolidine, imidazoline, piperidine, piperazine, indoline, morpholino, piperidinyl, tetrahydrofuranyl, and the like as well as N-alkoxy-nitrogen containing heterocycles.

A preferred class of heterocyclics include "crown compounds" which refers to a specific class of heterocyclic compounds having one or more repeating units of

20 the formula $[-(CH_2-)_m Y-]$ where m is ≥ 2, and Y at each separate occurrence can be O, N, S or P. Examples of crown compounds include, by way of example only, $[-(CH_2)_3-NH-]_3$, $[-((CH_2)_2-O)_4-((CH_2)_2-NH)_2]$ and the like. Typically such crown compounds can have from 4 to 10 heteroatoms and 8 to 40 carbon atoms.

The term "heterocyclooxy" refers to the group heterocyclic-O-.

25 The term "thioheterocyclooxy" refers to the group heterocyclic-S-.

The term "heterocyclene" refers to the diradical group formed from a heterocycle, as defined herein, and is exemplified by the groups 2,6-morpholino, 2,5-morpholino and the like.

The term "oxyacylamino" or "aminocarbonyloxy" refers to the group -OC(O)NRR where each R is independently hydrogen, alkyl, substituted alkyl, aryl, heteroaryl, or heterocyclic wherein alkyl, substituted alkyl, aryl, heteroaryl and heterocyclic are as defined herein.

The term "thiol" refers to the group -SH.

The term "thioalkoxy" refers to the group -S-alkyl.

The term "substituted thioalkoxy" refers to the group -S-substituted alkyl.

The term "thioaryloxy" refers to the group aryl-S- wherein the aryl group is as defined above including optionally substituted aryl groups also defined above.

The term "thioheteroaryloxy" refers to the group heteroaryl-S- wherein the heteroaryl group is as defined above including optionally substituted aryl groups as also defined above.

As to any of the above groups which contain one or more substituents, it is understood, of course, that such groups do not contain any substitution or substitution patterns which are sterically impractical and/or synthetically non-feasible. In addition, the compounds of this invention include all stereochemical isomers arising from the substitution of these compounds.

The term "pharmaceutically acceptable salt" refers to salts which retain the biological effectiveness and properties of the multibinding compounds of this invention and which are not biologically or otherwise undesirable. In many cases, the multibinding compounds of this invention are capable of forming acid and/or base salts by virtue of the presence of amino and/or carboxyl groups or groups similar thereto.

Pharmaceutically acceptable base addition salts can be prepared from inorganic and organic bases. Salts derived from inorganic bases, include by way of example only, sodium, potassium, lithium, ammonium, calcium and magnesium salts. Salts derived from organic bases include, but are not limited to, salts of primary, secondary and tertiary amines, such as alkyl amines, dialkyl amines, trialkyl amines, substituted alkyl amines, di(substituted alkyl) amines,

tri(substituted alkyl) amines, alkenyl amines, dialkenyl amines, trialkenyl amines, substituted alkenyl amines, di(substituted alkenyl) amines, tri(substituted alkenyl) amines, cycloalkyl amines, di(cycloalkyl) amines, tri(cycloalkyl) amines, substituted cycloalkyl amines, disubstituted cycloalkyl amine, trisubstituted

5   cycloalkyl amines, cycloalkenyl amines, di(cycloalkenyl) amines, tri(cycloalkenyl) amines, substituted cycloalkenyl amines, disubstituted cycloalkenyl amine, trisubstituted cycloalkenyl amines, aryl amines, diaryl amines, triaryl amines, heteroaryl amines, diheteroaryl amines, triheteroaryl amines, heterocyclic amines, diheterocyclic amines, triheterocyclic amines, mixed di- and tri-amines where at

10   least two of the substituents on the amine are different and are selected from the group consisting of alkyl, substituted alkyl, alkenyl, substituted alkenyl, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, heteroaryl, heterocyclic, and the like. Also included are amines where the two or three substituents, together with the amino nitrogen, form a heterocyclic or

15   heteroaryl group.

Examples of suitable amines include, by way of example only, isopropylamine, trimethyl amine, diethyl amine, tri(*iso*-propyl) amine, tri(*n*-propyl) amine, ethanolamine, 2-dimethylaminoethanol, tromethamine, lysine, arginine, histidine, caffeine, procaine, hydrabamine, choline, betaine,

20   ethylenediamine, glucosamine, N-alkylglucamines, theobromine, purines, piperazine, piperidine, morpholine, N-ethylpiperidine, and the like. It should also be understood that other carboxylic acid derivatives would be useful in the practice of this invention, for example, carboxylic acid amides, including carboxamides, lower alkyl carboxamides, dialkyl carboxamides, and the like.

25   Pharmaceutically acceptable acid addition salts may be prepared from inorganic and organic acids. Salts derived from inorganic acids include hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, and the like. Salts derived from organic acids include acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, malic acid, malonic acid, succinic acid,

maleic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid,
mandelic acid, methanesulfonic acid, ethanesulfonic acid, p-toluene-sulfonic acid,
salicylic acid, and the like.

The term "protecting group" or "blocking group" refers to any group which

5   when bound to one or more hydroxyl, thiol, amino or carboxyl groups of the
compounds (including intermediates thereof) prevents reactions from occurring at
these groups and which protecting group can be removed by conventional chemical
or enzymatic steps to reestablish the hydroxyl, thiol, amino or carboxyl group
Green[63]. The particular removable blocking group employed is not critical and

10   preferred removable hydroxyl blocking groups include conventional substituents
such as allyl, benzyl, acetyl, chloroacetyl, thiobenzyl, benzylidine, phenacyl, t-
butyl-diphenylsilyl and any other group that can be introduced chemically onto a
hydroxyl functionality and later selectively removed either by chemical or
enzymatic methods in mild conditions compatible with the nature of the product.

15   Preferred removable amino blocking groups include conventional
substituents such as t-butyoxycarbonyl (t-BOC), benzyloxycarbonyl (CBZ), and
the like which can be removed by conventional conditions compatible with the
nature of the product.

Preferred carboxyl protecting groups include esters such as methyl, ethyl,

20   propyl, t-butyl etc. which can be removed by mild hydrolysis conditions
compatible with the nature of the product.

The term "optional" or "optionally" means that the subsequently described
event, circumstance or substituent may or may not occur, and that the description
includes instances where said event or circumstance occurs and instances where it

25   does not.

As used herein, the terms "inert organic solvent" or "inert solvent" mean a
solvent inert under the conditions of the reaction being described in conjunction
therewith [including, for example, benzene, toluene, acetonitrile, tetrahydrofuran
("THF"), dimethylformamide ("DMF"), chloroform ($CHCl_3$), methylene chloride

(or dichloromethane or "CH_2Cl_2"), diethyl ether, ethyl acetate, acetone, methylethyl ketone, methanol, ethanol, propanol, isopropanol, tert-butanol, dioxane, pyridine, and the like]. Unless specified to the contrary, the solvents used in the reactions of the present invention are inert solvents.

5

A "receptor" or "cellular receptor" is a biological structure with one or more binding domains that reversibly complexes one or more ligands, where that complexation has biological consequences. Receptors are distinguished for the purpose of this application from enzymes, which bind and then transform the

10 bound species.

It should be recognized that the cellular receptors that participate in biological multivalent binding interactions are constrained to varying degrees by their intra- and intermolecular associations (e.g. cellular receptors may be covalently joined in a single structure, noncovalently associated in a multimeric

15 structure, embedded in a membrane or polymeric matrix and so on) and therefore have less translational and rotational freedom than if the same cellular receptors were present as monomers in solution.

The term "ligand binding site" as used herein denotes the site on a receptor that recognizes a ligand domain and provides a binding partner for that ligand.

20 The ligand binding site may be defined by monomeric or multimeric structures. This interaction may be capable of producing a unique biological effect, for example agonism, antagonism, modulatory effect and the like or may maintain an ongoing biological event. For the purposes of this description, the ligand, the ligand domain and the ligand binding site cannot both be DNA, RNA, an antibody,

25 an antibody domain or a fragment of an antibody.

"Ligand" as used herein denotes a compound that is a binding partner for a receptor and is bound thereto by complementarity. The specific region or regions of the ligand that is (are) recognized by the receptor is designated as the "ligand domain". A ligand may be either capable of binding to a receptor by itself, or

may require the presence of one or more non-ligand components for binding (e.g. Ca^{+2}, Mg^{+2} or a water molecule is required for the binding of a ligand domain to various receptors).

It is further understood that the term ligands is not intended to be limited to compounds known to be useful as receptor binding compounds (e.g., known drugs). It should be understood that portions of the ligand structure that are not essential for specific molecular recognition and binding activity may be varied substantially, replaced with unrelated structures and, in some cases, omitted entirely without affecting the binding interaction. The primary requirement for a ligand is that it has a ligand domain as defined above. Those skilled in the art will understand that the term ligand can equally apply to a molecule that is not normally associated with cellular receptor binding properties. In addition, it should be noted that ligands that exhibit marginal activity or lack useful activity as monomers can be highly active as multivalent compounds because of the benefits conferred by multivalency. The only requirement for a ligand is that it has a ligand binding domain as defined above.

Accordingly, examples of ligands useful for this invention are ligands for the muscarinic receptors; the α_2, β_1 and β_2 adrenergic receptors, the 5-HT receptors, the $GABA_A$ receptor, the melatonin receptor; the angiotensin I receptor; the erythropoietin receptor; the dopamine 1 and 2 receptors; the A2 adenosine receptor, the nicotinic receptors and the steroid receptor.

1.   Antagonists and agonists for muscarinic receptors include Bethanocol, Pilocarpine, Dicyclomine, Ipratropium (nonselective), Methoctramine (M1), Tiotropium (M3) and Pirenzipine (M1). These antagonists are used in the treatment of xerostomia, glaucoma, gastro-intestinal diseases, asthma, COPD, emesis, gastroprokinesis, memory enhancement, motion sickness, some symptoms of Parkinson's. Tiotropium is useful in decreasing bronchial secretions. Pirenzipine decreases gastric H+ secretions. Antagonists for the M1 muscarinic receptor are used to treat arrythmas, GERD and ulcers. Agonists for the M1

muscarinic receptor are useful in memory enhancement. Antagonists specifically for the M2 muscarinic receptor are useful in cardiac applications. Antagonists specifically for the M3 muscarinic receptor are used in urology. Finally, antagonists specifically for the M4 muscarinic receptor are useful in treating analgesia, arthritis, and central nervous system disorders such as neuroleptic conditions. The muscarinic receptor has the potential to accept a multivalent ligand because it has an additional allosteric site. Both the orthosteric and allosteric sites are on the extracellular portion of the receptor. This allows gallamine to bind with negative cooperativity to the cardiac M2 muscarinic receptor and alcuronium to bind with positive cooperativity to the M2 muscarinic receptor but with negative cooperativity to the M3 muscarinic receptor.

2.   Agonists for the opioid receptors include: (a) antagonists to the opioid κ receptor: buprenorphine and butorphanol; (b) antagonists to the opioid μ receptor: alfentanil, morphine, methadone, codeine, hydrocodone, hydromorphone, levorphanol, meperidine, nalbuphine, opium, oxycodone, oxymorphone, pentazocine, propoxyphene. These antagonists are useful in the treatment of pain, addiction and the provision of anesthesia. The opioid receptors lend themselves to the use of multimeric ligands because they form homodimers on the cell surface.

3.   Antagonists for the α_2 adrenergic receptor include clonidine and yohimbine. These compounds are used in the treatment of hypertension, hypertensive emergencies, headache, vascula, dysmenorrhea and menopause. The α_2 adrenergic receptor is amenable to multimeric compounds becauase it has an additional allosteric site in the receptor. For example, ameloride binds the receptor with negative cooperativity.

4.   Antagonists for the β_1 adrenergic receptor include atenolol. This compound is useful in the treatment of angina because it decreases workload of the heart and thus the oxygen consumption of the heart. The β_1 adrenergic receptor is amenable to multimeric ligands because it either dimerizes upon ligand binding or has two sites on one receptor.

5.   Agonists for the β_2 adrenergic receptor include albuterol, bitolterol, epinephrine, fenoterol, isoetharine, isoproterenol, metaproterenol, pirbuterol, procaterol, salmerterol, and terbutaline. These compounds are used in the treatment of asthma. The β_2 adrenergic receptor is amenable to multimeric ligands because it dimerizes upon ligand binding to be effective.

6.   Ligands for the 5-HT receptor include sumatriptan (binds the 5-HT$_{1B/1D}$ receptor); zolmitriptan (binds to the 5-HT$_{1B/1D}$ receptor, crosses the blood/brain barrier and complexes with the central nervous system vascular nucleus in addition to peripheral vascular sites); nefazodone (binds the 5-HT$_2$ receptor); risperidone (binds the 5-HT$_2$ receptor); mirtazapine (binds the 5-HT$_{2/3}$ receptors); granisetron (binds the 5-HT$_3$ receptor); ondansetron (binds the 5-HT$_3$ receptor); ondansetron (binds the 5-HT$_3$ receptor); paroxetine (binds all 5-HT receptors); and olanzapine (which binds the 5-HT$_2$ receptor and many D subtypes). These compounds are useful in the treatment of migraine headache, itch, depression, schizophrenia, and possibly motion sickness. Compounds which bind the 5-HT$_{1A}$ receptor are useful in treating depression and memory loss. Compounds which bind to the 5-HT$_{1B/D}$ receptor are useful in treating migraine. Compounds which bind to the 5-HT$_{1D/E/F}$ receptors are useful in treating anxiolytic disorders and obesity. The ligands for the 5-HT receptors will be useful in the multivalomeric states because the receptor dimerizes upon ligand binding and allosterism is likely with various synthetic ligands, such as methysergide.

7.   Antagonists for the NMDA receptor include felbamate. This compound is useful in providing neuroprotection following a stroke. It is also an antiepileptic and an anticonvulsant. The NMDA receptor contains multiple sites plus allosteric sties and accordingly, is amenable to multivalomeric ligands of the present invention.

8.   Agonists for the GABA$_a$ receptor include benzodiazepines and barbituates. These compounds are useful as anxiolytics, hypnotics, anticonvulsants, muscle

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relaxants, and anterograde amnestics. The GABA receptor contains multiple known binding sites for different small molecules.

9.   Agonists for the melatonin receptor include melatonin. This compound is useful in treating sleep disorders and maintaining a sense of well-being in the patient. The melatonin receptor has two sites for melatonin.

10.   Antagonists for the angiotensin 2 receptor include losartan and eprosartan. These compounds are useful in the treatment of hypertension. Angiotensin I binds to the angiotensin I receptor twice whereas Losartan only binds the receptor once. The binding sites are believed to be on opposite sides of the cell membrane. It would be advantageous to develop a multivalomeric antagonist that was able to bind to a single receptor twice.

11.   Agonists for the erythropoietin receptor include Epogen. This compound is useful in the treatment of anemia, especially secondary anemia due to decreased renal production of erythropoietin. This receptor dimerizes upon ligand binding.

12.   Agonists for the dopamine 1 receptor include dopamine. This compound is useful in treating Parkinson's disease and is used as a vasoactive in the intensive care unit. The dopamine 1 receptor also dimerizes upon ligand binding.

13.   Antagonists for the dopamine 2 receptor include metoclopromide and haloperidol. These compounds are useful for appetite enhancement, dyskinesis, Huntington's chorea and emesis. The dopamine 2 receptor also dimerizes upon ligand binding and ameloride is known to bind at an allosteric site.

14.   Agonists for the A_2 adenosine receptor include adenosine. This compound acts as an anti-arrhythmic during imaging and echo diagnostics. The A1 adenosine receptor has an additional allosteric site. It has been found that aminobenzythiopenes bind with positive cooperativity to the A1 adenosine receptor.

15.   Ligands to the nicotinic receptor include succinylcholine. This comound is useful in the treatment of paralysis and myasthenia gravis. It can be used as a muscle relaxant. The nicotinic receptor has two sites for the ligand per receptor.

16.   Ligands for the steroid receptor include (a) the corticosteroids: beclomethasone, budesonide, dexamethasone, flunisolide and triamcinolone; (b) the progestins: hydroyprogesterone, levonorgestrel, medrogestone, medroxyprogesterone, megestrol, norethinidrone, norgestrel and progesterone; (c) the estrogens: estrogen, tamoxifen and raloxifene; (d) the anabolic steroids; and (e) the mineralacorticoids. These compound are useful for a range of diseases from inflammation to cancer. The steroid receptor dimerizes upon binding of the ligand.

The ligands and linkers which comprise the multibinding agents of the invention may have various stereoisomeric forms, including enantiomers and diastereomers.

"Multi-binding agents" or "multi-binding compounds" refers to a compound that is capable of multi-valency as defined below, and which has 2 -10 ligands covalently bound to one or more linkers which may be the same or different. In all cases, each ligand and linker in the multibinding compound is independently selected such that the multibinding compound includes both symmetric compounds (i.e. where each ligand as well as each linker is identical) and asymmetric compounds (i.e. where at least one of the ligands is different from the other ligand(s) and/or at least one linker is different from the other linker(s). Additionally, the term is intended to include the racemic forms of the multibinding compound as well as individual enantiomers and diastereomers and non-racemic mixtures thereof. It is to be understood that the invention contemplates all possible stereoisomeric forms of multibinding compounds and mixtures thereof. A multibinding agent provides a biological and/or therapeutic effect greater than the aggregate of unlinked ligands equivalent thereto which may be the same or different. That is to say that the biological and/or therapeutic effect of the ligands attached to the multi-binding compound is greater than that achieved by the same amount of unlinked ligands made available for binding to the ligand binding sites.

It should be further understood that multibinding compounds may exhibit intrinsically new and desirable activities relative to their component ligands. For

example, appropriate linking of receptor agonists and/or antagonists may provide compounds which display partial agonist properties. It should also be understood that the overall potency and degree of efficacy may be controlled and selected through choice of agonist ligands, antagonist ligands and linkers. By way of

5 example, the joining of one or more β_2 adrenergic receptor agonists with one or more β_2 adrenergic receptor antagonists provides a compound which is a potent agonist of β_2 adrenergic receptors but with sub-maximal efficacy (i.e., it is a partial agonist). In this instance, a partial agonist is preferred in order to avoid cardiovascular side effects and tachyphylaxis.

10 The phrase "increased biological or therapeutic effect" includes, for example increased affinity for a target, increased specificity for a target, increased selectivity for a target, increased potency, increased efficacy, decreased toxicity, improved duration of action, decreased side effects, increased therapeutic index, improved bioavailability, improved pharmacokinetics, improved activity spectrum,

15 and the like. The multi-binding compounds of this invention will exhibit at least one and preferably more than one of the above mentioned effects.

"Uni-valency" as used herein refers to a single binding interaction between one ligand as defined herein with one ligand binding site as defined herein. It should be noted that a molecule having multiple copies of a ligand (or ligands)

20 exhibits univalency when only one ligand is interacting with a ligand binding site. Examples of univalent interactions are depicted below.
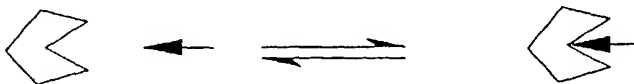
25 "Multi-valency" as used herein refers to the concurrent binding of from 2 to 10 linked ligands (which may be the same or different) and two or more corresponding ligand binding sites on the receptors which receptors may be the same or different.

For example, two ligands connected by a linker that bind concurrently to two ligand binding sites would be considered as bi-valency; three ligands thus connected would be an example of tri-valency. An example of tri-valency illustrating a multi-binding agent bearing 3 ligands versus a monovalent binding

5   interaction is shown below.
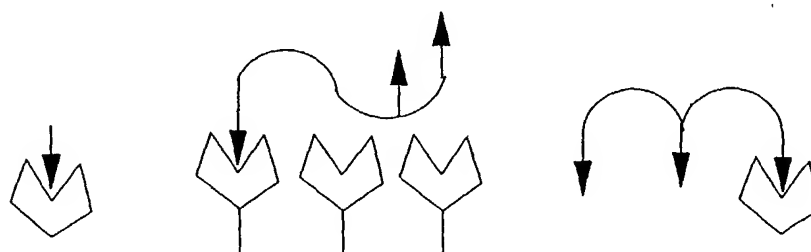
univalent interactions
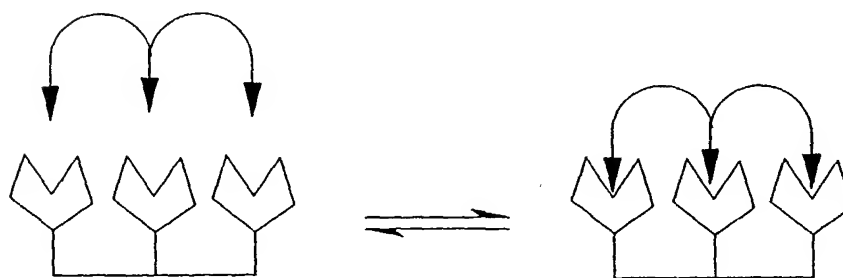
multivalent interaction

It should be understood that all compounds that contain multiple copies of a ligand attached to a linker do not necessarily exhibit the phenomena of multi-

25   valency, i.e., that the biological and/or therapeutic effect of the multi-binding agent is greater than the sum of the aggregate of unlinked ligands made available to the ligand binding site. For multi-valency to occur, the ligands that are connected by a linker have to be presented to their receptors by the linker in a specific manner in

order to bring about the desired ligand-orienting result, and thus produce a multi-binding agent.

"Potency" as used herein refers to the minimum concentration at which a ligand is able to achieve a desirable biological or therapeutic effect. The potency of a ligand is typically proportional to its affinity for its ligand binding site. In some cases the potency may be non-linearly correlated with its affinity. In comparing the potency of two drugs, e.g., a multi-binding agent and the aggregate of its unlinked ligand, the dose-response curve of each is determined under identical test conditions (e.g. an *in vitro* or *in vivo* assay, in an appropriate animal model such as a human patient). The finding that the multi-binding agent produces an equivalent biological or therapeutic effect at a lower concentration than the aggregate unlinked ligand (e.g. on a per weight, per mole or per ligand basis) is indicative of enhanced potency.

"Selectivity" or "specificity" is a measure of the binding preferences of a ligand for different ligand binding sites. The selectivity of a ligand with respect to its target ligand binding site relative to another ligand binding site is given by the ratio of the respective values of K_d (i.e., the dissociation constants for each ligand-receptor complex) or in cases where a biological effect is observed below the K_d, the ratio of the respective EC_{50} s (i.e., the concentrations that produce 50% of the maximum reponse for the ligand interacting with the two distinct ligand binding sites

The terms "agonism" and "antagonism" are well known in the art. The term "modulatory effect" refers to the ability of the ligand to change the activity of an agonist or antagonist through binding to a ligand binding site.

The term "treatment" refers to any treatment of a pathologic condition in a mammal or bird, particularly a human, and includes:

(i)   preventing the pathologic condition from occurring in a subject which may be predisposed to the condition but has not yet been diagnosed with the

condition and, accordingly, the treatment constitutes prophylactic treatment for the disease condition;

   (ii) inhibiting the pathologic condition, i.e., arresting its development;

   (iii) relieving the pathologic condition, i.e., causing regression of the pathologic condition; or

   (iv) relieving the conditions mediated by the pathologic condition, e.g., relieving the conditions caused by an enterotoxin expressed by a microorganism but not addressing the underlining microbial infection.

The term "pathologic condition which is modulated by treatment with a ligand" covers all disease states (i.e., pathologic conditions) which are generally acknowledged in the art to be usefully treated with a ligand for a cellular receptor in general, and those disease states which have been found to be usefully treated by a specific multi-binding compound. Such disease states include, by way of example only, the treatment of a mammal afflicted with migraine headache, depression, hypertension and the like. It also covers the treatment of pathologic conditions that are not necessarily generally considered as pathologic conditions, for example the use of multi-binding compounds in the treatment of pregnancy, obesity, hair-loss, beauty aids and the like.

The term "therapeutically effective amount" refers to that amount of multi-binding compound which is sufficient to effect treatment, as defined above, when administered to a mammal or bird in need of such treatment. The therapeutically effective amount will vary depending upon the subject and disease condition being treated, the weight and age of the subject, the severity of the disease condition, the manner of administration and the like, which can readily be determined by one of ordinary skill in the art.

The term "linker", identified where appropriate by the symbol X, refers to a group or groups that covalently link(s) from 2 to 10 ligands (as identified above) in a manner that provides for a compound capable of multi-valency when in the presence of at least one cellular receptor having 2 or more ligand binding sites.

The linker is a ligand-orienting entity that permits attachment of multiple copies of a ligand (which may be the same or different) thereto. The linker may be either a chiral or an achiral molecule. In some cases the linker may be biologically active. The term linker does not, however, extend to cover solid inert supports such as

5   beads, glass particles, fibers and the like. But it is to be understood that the multi-binding compounds of this invention can be attached to a solid support if desired, for example, for use in separation and purification processes and for similar applications.

The extent to which multivalent binding is realized depends upon the

10   efficiency with which the linker or linkers that joins the ligands presents them to their ligand binding sites on one or more receptors. Beyond presenting ligands for multivalent interactions with ligand binding sites, the linker spatially constrains these interactions to occur within dimensions defined by the linker. Thus the structural features of the linker (valency, geometry, orientation, size, flexibility,

15   chemical composition) are features of multivalent compounds that play an important role in determining their activities.

The linkers used in this invention are selected to allow multivalent binding of ligands to any desired ligand binding sites of a receptor, whether such sites are located interiorly, both interiorly and on the periphery of the molecule, or at any

20   intermediate position thereof. The distance between the nearest neighboring ligand domains is preferably in the range of about 2Å to about 100Å, more preferably in the range of about 3Å to about 40Å.

The ligands are covalently attached to the linker or linkers using conventional chemical techniques providing for covalent linkage of the ligand to the

25   linker or linkers. The reaction chemistries resulting in such linkages are well known in the art and involve the use of complementary functional groups on the linker and ligand. Preferably, the complementary functional groups on the linker are selected relative to the functional groups available on the ligand for binding or which can be introduced onto the ligand for binding. Again, such complementary

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functional groups are well known in the art. For example, reaction between a carboxylic acid of either the linker or the ligand and a primary or secondary amine of the ligand or the linker in the presence of suitable well-known activating agents results in formation of an amide bond covalently linking the ligand to the linker; reaction between an amine group of either the linker or the ligand and a sulfonyl halide of the ligand or the linker results in formation of a sulfonamide bond covalently linking the ligand to the linker; and reaction between an alcohol or phenol group of either the linker or the ligand and an alkyl or aryl halide of the ligand or the linker results in formation of an ether bond covalently linking the ligand to the linker.

The following table illustrates numerous complementary reactive groups and the resulting bonds formed by reaction there between. Where functional groups are lacking, they can be created by suitable chemistries that are described in standard organic chemistry texts such as J. March[1].

COMPLEMENTARY BINDING CHEMISTRIES

FIRST REACTIVE GROUP	SECOND REACTIVE GROUP	LINKAGE
hydroxyl	isocyanoate	urethane
amine	epoxide	amine/alcohol
tosyl halide	amine	sulfonamide
carboxyl	amine	amide
hydroxyl	alkyl/aryl halide	ether

The linker is attached to the ligand at a position that retains ligand binding domain-receptor binding and specifically which permits the receptor recognition site of the ligand to orient itself to bind to the receptor. Such positions and synthetic protocols for linkage are well known in the art. Following attachment to

the linker or a significant portion thereof (e.g. 2-10 atoms of linker), the linker-ligand conjugate is tested for retention of activity in a relevant assay system. If a linker-ligand conjugate shows activity at a concentration of less than 1 mM, it is considered to be acceptable for use in constructing a multi-binding compound. The relative orientation in which the ligand domains are displayed to the receptors depends both on the particular point (or points) of attachment of the ligands to the linker, and on the framework geometry. The term linker embraces everything that is not considered to be part of the ligand.

Suitable linkers are discussed below.

At present, it is preferred that the multi-binding agent is a bivalent compound, e.g., two ligands which are covalently linked to linker X.

The term "library" refers to at least 3, preferably from 10^2 to 10^9 and more preferably from 10^2 to 10^4 multimeric compounds. Preferably, these compounds are prepared as a multiplicity of compounds in a single solution or reaction mixture which permits facile synthesis thereof. In one embodiment, the library of multimeric compounds can be directly assayed for multibinding properties. In another embodiment, each member of the library of multimeric compounds is first isolated and, optionally, characterized. This member is then assayed for multibinding properties.

The term "collection" refers to a set of multimeric compounds which are prepared either sequentially or concurrently (e.g., combinatorially). The collection comprises at least 2 members; preferably from 2 to 10^9 members and still more preferably from 10 to 10^4 members.

The term "multimeric compound" refers to compounds comprising from 2 to 10 ligands covalently connected through at least one linker which compounds may or may not possess multibinding properties (as defined herein).

The term "pseudohalide" refers to functional groups which react in displacement reactions in a manner similar to a halogen. Such functional groups include, by way of example, mesyl, tosyl, azido and cyano groups.

Methodology

The linker, when covalently attached to multiple copies of the ligands, provides a biocompatible, substantially non-immunogenic multi-binding compound. The biological activity of the multi-binding compound is highly sensitive to the

5 valency, geometry, composition, size, flexibility or rigidity, etc. of the linker as well as the presence or absence of anionic or cationic charge, the relative hydrophobicity/hydrophilicity of the linker, and the like on the linker. Accordingly, the linker is preferably chosen to maximize the biological activity of the multi-binding compound. The linker may be biologically "neutral", i.e., not

10 itself contribute any biological activity to the multi-binding compound or it may be chosen to enhance the biological activity of the molecule. In general, the linker may be chosen from any organic molecule construct that orients two or more ligands to the receptors to permit multivalency. In this regard, the linker can be considered as a "framework" on which the ligands are arranged in order to bring

15 about the desired ligand-orienting result, and thus produce a multi-binding compound.

For example, different orientations can be achieved by including in the framework groups containing mono- or polycyclic groups, including aryl and/or heteroaryl groups, or structures incorporating one or more carbon-carbon multiple

20 bonds (alkenyl, alkenylene, alkynyl or alkynylene groups). Other groups can also include oligomers and polymers which are branched- or straight-chain species. In preferred embodiments, rigidity is imparted by the presence of cyclic groups (e.g., aryl, heteroaryl, cycloalkyl, heterocyclic, etc.) In other preferred embodiments, the ring is a six or ten member ring. In still further preferred embodiments, the ring is

25 an aromatic ring such as, for example, phenyl or naphthyl.

Different hydrophobic/hydrophilic characteristics of the linker as well as the presence or absence of charged moities can readily be conducted by the skilled artisan. For example, the hydrophobic nature of a linker derived from hexamethylene diamine ($H_2N(CH_2)_8NH_2$ or related polyamines can be modified to

be substantially more hydrophilic by replacing the alkylene group with a

poly(oxyalkylene) group such as found in the commercially available "Jeffamines".

Different frameworks can be designed to provide preferred orientations of

the ligands. Such frameworks may be represented by using an array of dots (as

5   shown below) wherein each dot may potentially be an atom, such as C, O, N, S, P,

H, F, Cl, Br, and F or the dot may alternatively indicate the absence of an atom at

that position. To facilitate the understanding of the framework structure, the

framework is illustrated as a two dimensional array in the following diagram,

although clearly the framework is a three dimensional array in practice:

```
       :   :        .     :         :    .
   8   ▪   ▪   ▪   ▪   ▪   ▪   ▪   ▪   ▪   .....
   7   ▪   ▪   ▪   ▪   ▪   ▪   ▪   ▪   ▪   .....
   6   ▪   ▪   ▪   ▪   ▪   ▪   ▪   ▪   ▪   .....
   5   ▪   ▪   ▪   ▪   ▪   ▪   ▪   ▪   ▪   .....
   4   ▪   ▪   ▪   ▪   ▪   ▪   ▪   ▪   ▪   ......
   3   ▪   ▪   ▪   ▪   ▪   ▪   ▪   ▪   ▪   .....
   2   ▪   ▪   ▪   ▪   ▪   ▪   ▪   ▪   ▪   .....
   1   ▪   ▪   ▪   ▪   ▪   ▪   ▪   ▪   ▪   .....
   0   ▪   ▪   ▪   ▪   ▪   ▪   ▪   ▪   ▪   .....
       0   1   2   3   4   5   6   7   8
```
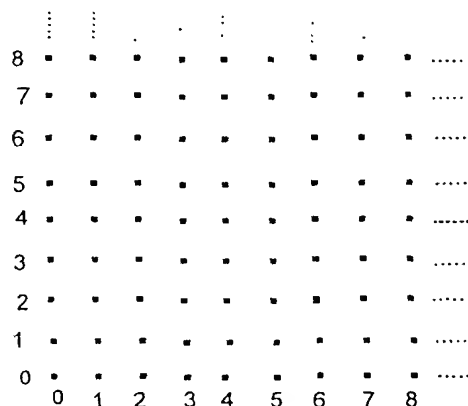
10   Each dot is either an atom, chosen from carbon, hydrogen, oxygen,

nitrogen, sulfur, phosphorus, or halogen, or the dot represents a point in space

(i.e., an absence of an atom). Only certain atoms on the grid have the ability to act

as an attachment point for the ligands, namely, C, O, N, S and P.

Atoms can be connected to each other via bonds (single, double or triple

15   bonds with acceptable resonance and tautomeric forms), with regard to the usual

constraints of chemical bonding. Ligands may be attached to the framework via

single, double or triple bonds (with chemically acceptable tautomeric and resonance

forms). Multiple ligand groups (2 to 10) can be attached to the framework such

that the minimal, shortest path distance between adjacent ligand groups does not

exceed 100 atoms. Preferably, the linker connections to the ligand is selected such that the maximum spatial distance between two adjacent ligands is no more than 40 Angstroms (Å).

An example of a linker as presented by the grid is shown below for a
5   biphenyl construct.
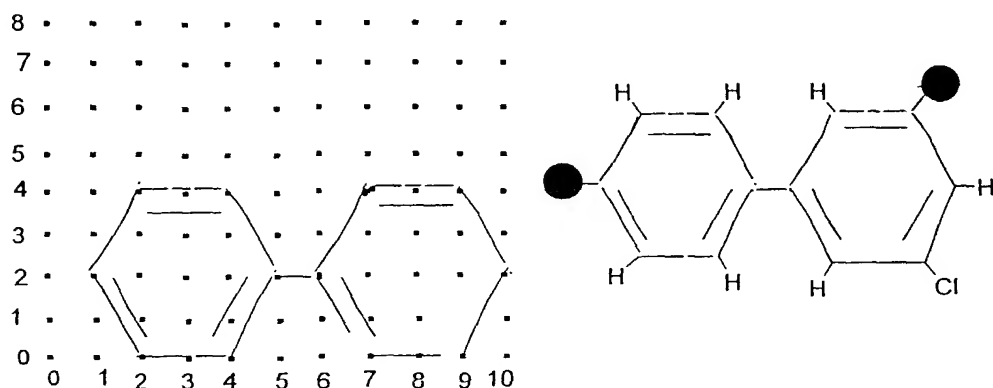
Nodes (1,2), (2,0), (4,4), (5,2), (4,0), (6,2), (7,4), (9,4), (10,2), (9,0), (7,0) all represent carbon atoms. Node (10,0) represents a chlorine atom). All other nodes
10   (or dots) are points in space (i.e., represent an absence of atoms.

Nodes (1,2) and (9,4) are attachment points.

Hydrogen atoms are affixed to nodes (2,4), (4,4), (4,0), (2,0), (7,4), (10,2) and (7,0).

Nodes (5,2) and (6,2) are connected by a single bond.

15   The carbon atoms present are connected by either a single or double bonds, taking into consideration the principle of resonance and/or tautomerism.

The intersection of the framework (linker) and the ligand group, and indeed, the framework (linker) itself can have many different bonding patterns. Examples of acceptable patterns of three contiguous atom arrangements are shown in the
20   following diagram:

CCC	NCC	OCC	SCC	PCC
CCN	NCN	OCN	SCN	PCN
CCO	NCO	OCO	SCO	PCO
CCS	NCS	OCS	SCS	PCS
CCP	NCP	OCP	SCP	PCP
CNC	NNC	ONC	SNC	PNC
CNN	NNN	ONN	SNN	PNN
CNO	NNO	ONO	SNO	PNO
CNS	NNS	ONS	SNS	PNS
CNP	NNP	ONP	SNP	PNP
COC	NOC	OOC	SOC	POC
CON	NON	OON	SON	PON
COO	NOO	OOO	SOO	POO
COS	NOS	OOS	SOS	POS
COP	NOP	OOP	SOP	POP
CSC	NSC	OSC	SSC	PSC
CSN	NSN	OSN	SSN	PSN
CSO	NSO	OSO	SSO	PSO
CSS	NSS	OSS	SSS	PSS
CSP	NSP	OSP	SSP	PSP
CPC	NPC	OPC	SPC	PPC
CPN	NPN	OPN	SPN	PPN
CPO	NPO	OPO	SPO	PPO
CPS	NPS	OPS	SPS	PPS
CPP	NPP	OPP	SPP	PPP

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One skilled in the art would be able to identify bonding patterns that would produce multivalent compounds. Methods for producing these bonding arrangements are described in March[1]. These arrangements are described in the grid of dots shown in the scheme above. All of the possible arrangements for the five most preferred atoms are shown. Each atom has a variety of acceptable oxidation states. The bonding arrangements underlined are less acceptable and are not preferred.

Examples of molecular structures in which the above bonding patterns could be employed as components of the linker are shown below.
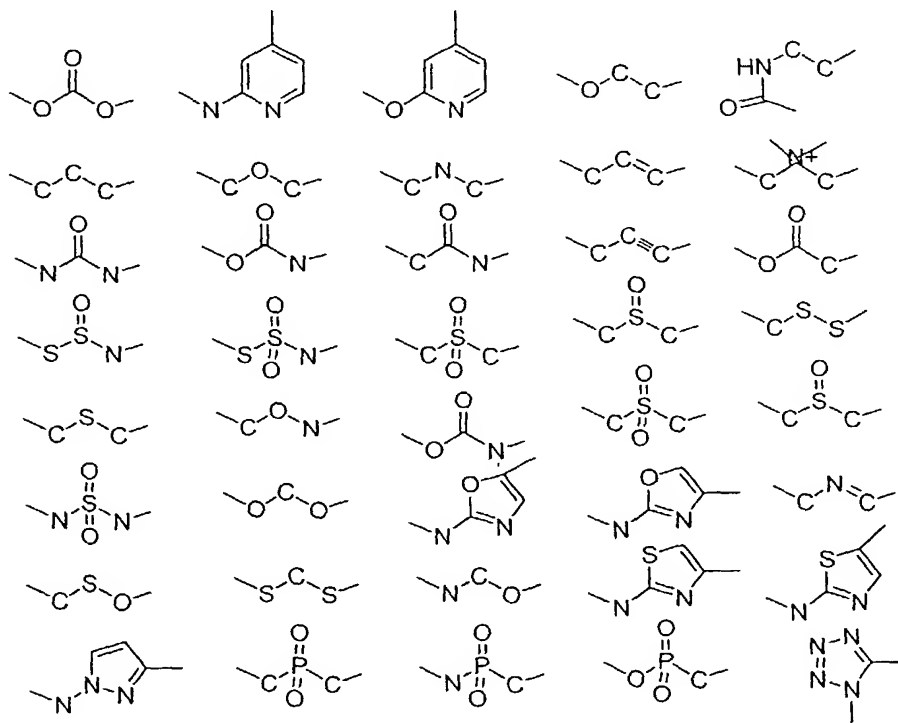
The identification of an appropriate framework geometry for ligand domain presentation is an important first step in the construction of a multivalent binding

agent with enhanced activity. Systematic spatial searching strategies can be used to aid in the identification of preferred frameworks through an iterative process.

An example of this process for extending the framework (linker) from the ligand is presented below for the triptan class of pharmaceuticals. Some of the possible locations for elaboration into the framework (linker) are shown in Figures 16 and 18 with arrows and "M". Some of the possible locations for elaboration into the framework for the tropane core compounds are shown in Figure 25.

Examples of triptan bivalent compounds are shown in Figures 19 - 23.

It can therefore be seen that there is a plethora of possibilities for the composition of a linker. Examples of linkers include aliphatic moieties, aromatic moieties, steroidal moieties, peptides, and the like. Specific examples are peptides or polyamides, hydrocarbons, aromatic groups, ethers, lipids, cationic or anionic groups, or a combination thereof. A wide diversity of linkers is commercially available from Chemsources USA; ChemSources International and ACD. Many of the linkers that are suitable for use in this invention fall into this category. Others can be readily synthesized by methods known in the art and described below.

Assay of each of the individual compounds of a collection generated as described above will lead to a subset of compounds with the desired enhanced activities (e.g. potency, selectivity). The analysis of this subset using a technique such as Ensemble Molecular Dynamics will provide a framework orientation that favors the properties desired. Having selected a preferred framework geometry, the physical properties of the linker can be optimized by varying the chemical composition. The composition of a linker can be varied in numerous ways to achieve the desired physical properties.

Examples are given below, but it should be understood that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. For example, properties of the linker can be modified by the addition or insertion of ancillary groups into the linker, for example, to change the solubility of the multi-binding compound (in water, fats,

lipids, biological fluids, etc.), hydrophobicity, hydrophilicity, linker flexibility, antigenicity, stability, and the like. For example, the introduction of one or more poly(ethylene glycol) (PEG) groups onto the linker enhances the hydrophilicity and water solubility of the multi-binding compound, increases both molecular weight and molecular size and, depending on the nature of the unPEGylated linker, may increase the *in vivo* retention time. Further PEG decreases antigenicity and potentially enhances the overall rigidity of the linker.

Ancillary groups which enhance the water solubility/hydrophilicity of the linker and, accordingly, the resulting multi-binding compounds are useful in practicing this invention. Thus, it is within the scope of the present invention to use ancillary groups such as, for example, poly(ethylene glycols), alcohols, polyols, (e.g., glycerin, glycerol propoxylate, saccharides, including mono-, oligo- and polysaccharides, etc.) carboxylates, polycarboxylates, (e.g., polyglutamic acid, polyacrylic acid, etc.), amines, polyamines, (e.g., polylycine, poly(ethyleneimine), and the like) to enhance the water solubility and/or hydrophilicity of the multi-binding compounds of this invention. In preferred embodiments, the ancillary group used to improve water solubility/hydrophilicity will be a polyether. In particularly preferred embodiments, the ancillary group will be a poly(ethylene glycol).

The incorporation of lipophilic ancillary groups within the structure of the linker to enhance the lipophilicity and/or hydrophobicity of the multi-binding compounds described herein is within the scope of this invention. Lipophilic groups useful with the linkers of this invention include, by way of example only, aryl and heteroaryl groups which, as above, may be either unsubstituted or substituted with other groups, but are at least substituted with a group which allows their covalent attachment to the linker. Other lipophilic groups useful with the linkers of this invention include fatty acid derivatives which do not form bilayers in aqueous medium until higher concentrations are reached.

Also within the scope of this invention is the use of ancillary groups which result in the multi-binding compound being incorporated into a vesicle such as a liposome or a micelle. The term "lipid" refers to any fatty acid derivative that is capable of forming a bilayer such that a hydrophobic portion of the lipid material orients toward the bilayer while a hydrophilic portion orients toward the aqueous phase. Hydrophilic characteristics derive from the presence of phosphato, carboxylic, sulfato, amino, sulfhydryl, nitro and other like groups well known in the art. Hydrophobicity could be conferred by the inclusion of groups that include, but are not limited to, long chain saturated and unsaturated aliphatic hydrocarbon groups of up to 20 carbon atoms and such groups substituted by one or more aryl, heteroaryl, cycloalkyl, and/or heterocyclic group(s). Preferred lipids are phosphoglycerides and sphingolipids, representative examples of which include phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, phosphatidic acid, palmitoyleoyl phosphatidylcholine, lysophosphatidylcholine, lysophosphatidyl-ethanolamine, dipalmitoylphosphatidylcholine, dioleoylphosphatidylcholine, distearoyl-phosphatidylcholine or dilinoleoylphosphatidylcholine could be used. Other compounds lacking phosphorus, such as sphingolipid and glycosphingolipid families are also within the group designated as lipid. Additionally, the amphipathic lipids described above may be mixed with other lipids including triglycerides and sterols.

The flexibility of the linker can be manipulated by the inclusion of ancillary groups which are bulky and/or rigid. The presence of bulky or rigid groups can hinder free rotation about bonds in the linker or bonds between the linker and the ancillary group(s) or bonds between the linker and the functional groups. Rigid groups can include, for example, those groups whose conformational lability is restrained by the presence of rings and/or multiple bonds, for example, aryl, heteroaryl, cycloalkyl and heterocyclic groups. Other groups which can impart rigidity include polypeptide groups such as oligo- or polyproline chains.

Rigidity can also be imparted electrostatically. Thus, if the ancillary groups are either positively or negatively charged, the similarly charged ancillary groups will force the presenter linker into a configuration affording the maximum distance between each of the like charges. The energetic cost of bringing the like-charged groups closer to each other will tend to hold the linker in a configuration that maintains the separation between the like-charged ancillary groups. Further ancillary groups bearing opposite charges will tend to be attracted to their oppositely charged counterparts and potentially may enter into both inter- and intramolecular ionic bonds. This non-covalent mechanism will tend to hold the linker into a conformation which allows bonding between the oppositely charged groups. The addition of ancillary groups which are charged, or alternatively, bear a latent charge when deprotected, following the addition to the linker, include deprotectation of a carboxyl, hydroxyl, thiol or amino protecting group, by a change in pH, oxidation, reduction or other mechanisms known to those skilled in the art, is within the scope of this invention.

Bulky groups can include, for example, large atoms, ions (e.g., iodine, sulfur, metal ions, etc.) or groups containing large atoms, polycyclic groups, including aromatic groups, non-aromatic groups and structures incorporating one or more carbon-carbon multiple bonds (i.e., alkenes and alkynes). Bulky groups can also include oligomers and polymers which are branched- or straight-chain species. Species that are branched are expected to increase the rigidity of the structure more per unit molecular weight gain than are straight-chain species.

In preferred embodiments, rigidity is imparted by the presence of cyclic groups (e.g., aryl, heteroaryl, cycloalkyl, heterocyclic, etc.). In still further preferred embodiments, the ring is an aryl group such as, for example, phenyl or naphthyl. In other preferred embodiments, the linker comprises one or more six-membered rings or crown groups which, while not rigid, retain the conformation of the linker through conformational entropy.

In view of the above, it is apparent that the appropriate selection of a linker group providing suitable orientation, entropy and physico-chemical properties is well within the skill of the art. Eliminating or reducing antigenicity of the multibinding compounds described herein is also within the scope of this invention.

As explained above, the multi-binding compounds described herein comprise 2-10 ligands attached to a linker that links the ligands in such a manner that they are presented to the cellular receptor for multivalent interactions. The linker spatially constrains these interactions to occur within dimensions defined by the linker, thus greatly increasing biological activity of the multi-binding compound as compared to the same number of ligands used in mono-binding form.

The multi-binding compounds of this invention are preferably represented by the empirical formula $(L)_p(X)_q$ where L, X, p and q are as defined above. This is intended to include the several ways in which the ligands can be linked together in order to achieve the objective of multivalency, and a more detailed explanation is described below.

As noted previously, the linker may be considered as a framework to which ligands are attached. Thus, it should be recognized that the ligands can be attached at any suitable position on this framework, for example, at the termini of a linear chain or at any intermediate position.

The simplest and most preferred multi-binding compound is a bivalent compound which can be represented as L-X-L, where L is a ligand and is the same or different and X is the linker. Examples of such bivalent compounds is provided in Figure 12. A trivalent compound could also be represented in a linear fashion, i.e., as a sequence of repeated units L-X-L-X-L, in which L is a ligand and is the same or different at each occurrence, as can X. However, a trimer can also be a multibinding compound comprising three ligands attached to a central core, and thus represented as $(L)_3X$, where the linker X could include, for example, an aryl or cycloalkyl group. Illustrations of trivalent and tetravalent compounds of this

invention are found in Figures 13 and 14 respectively. Tetravalent compounds can be represented as

$$L\text{-}X\text{-}L\text{-}X\text{-}L\text{-}X\text{-}L$$
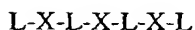
or in a branched array, e.g.,

$$
\begin{array}{c}
L\text{-}X\text{-}L\text{-}X\text{-}L \\
| \\
L
\end{array}
$$
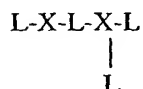
(a branched construct analogous to the isomers of butane -- *n*-butyl, *iso*-butyl, *sec*-butyl, and *t*-butyl)or in a tetrahedral array, e.g.,
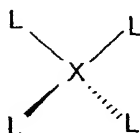
where X and L are as defined herein. Alternatively, it could be represented as an aryl or cycloalkyl derivative as above with four (4) ligands attached to the core linker.

The same considerations apply to higher multi-binding compounds of this invention containing 5-10 ligands as illustrated in Figure 15. However, for multibinding agents attached to a central linker such as aryl or cycloalkyl, there is a self-evident constraint that there must be sufficient attachment sites on the linker to accommodate the number of ligands present; for example, a benzene ring could not accommodate more than 6 ligands, whereas a multi-ring linker (e.g., biphenyl) could accommodate a larger number of ligands.

Certain of the above described compounds may alternatively be represented as cyclic chains of the form:

$$\begin{array}{c} \overset{\displaystyle L}{\overset{\displaystyle \frown}{}} \\ X \qquad X \\ \underset{\displaystyle L}{\underset{\displaystyle \smile}{}} \end{array}$$
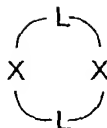
and variants thereof.

All of the above variations are intended to be within the scope of the invention defined by the formula $(L)_p(X)_q$.

With the foregoing in mind, preferred linkers may be represented by the following formula:

$$-X'-Z-(Y'-Z)_m-Y''-Z-X'-$$
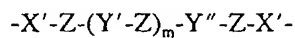
in which:

m is an integer of from 0 to 20;

X' at each separate occurrence is selected from the group consisting of -O-, -S-, -NH-, -C(O)-, -C(O)O-, -C(O)NH-, -C(S), -C(S)O-, -C(S)NH- or a covalent bond;

Z is at each separate occurrence is selected from the group consisting of alkylene, cycloalkylene, alkenylene, alkynylene, arylene, heteroarylene, heterocyclene, or a covalent bond;

Y' and Y'' at each separate occurrence are selected from the group consisting of

-S(O)$_n$-CR'R"-

-S(O)$_n$-NR'-
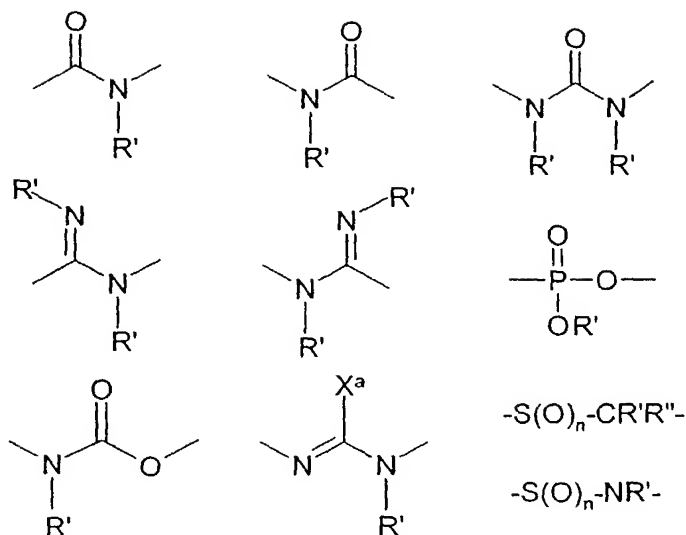
-S-S- or a covalent bond;

in which:

n is 0, 1 or 2; and

R, R' and R" at each separate occurrence are selected from the group consisting of hdyrogen, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, heteroaryl and heterocyclic.

Additionally, the linker moiety can be optionally substituted at any atom therein by one or more alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, heteroaryl and heterocyclic group.

Accordingly, compounds of Formula I may be prepared as shown below.

As indicated above, the simplest (and preferred) construct is a bivalent compound which can be represented as L-X-L, where L is a ligand and is the same or different, and X is the linker.

Accordingly, an example of the preparation of a bivalent ligand is given below as an illustration of the manner in which multivalent compounds of Formula I are obtained. This example is applicable to any ligand that includes amino and/or carboxyl groups and examples of different linkers (X) are shown. In the reactions schemes that follow, for ease of understanding of the principles involved, the structure of the ligand is presented as a "box". Thus, the ligand is illustrated such that carboxyl [C], amino [V], and methylamino [N] groups are shown as examples.

Preparation of Compounds of Formula I

Accordingly, compounds of Formula I may be prepared as shown as below.

As indicated above, the simplest (and preferred) construct is a bivalent compound, which can be represented L-X-L, where L is a ligand and is the same or different at each occurrence, and X is the linker.

Two ligands are connected by the linker X via carboxyl group or amino group of a first ligand, as indicated as R^1, to any carboxyl group or amino group of a second ligand, indicated as R^2.

Another simplification in the description of the preparations is that, for example, compound (1) is illustrated as a compound of formula $H_2N-(CH_2)_m$-$NCHO_2$-t-butyl, in which m is an integer of 1-20. However, it should be understood that $(CH_2)_m$ is not intended to signify or imply that the scope of this reaction (or of the invention) is limited to straight (i.e. unbranched) alkylene chains, but rather $(CH_2)_m$ is intended to include branched alkylenes as defined above, and alkylenes optionally substituted by aryl, arylalkyl, heteroaryl, heteroarylalkyl, and the like, also as disclosed in the Detailed Description of the Invention. Similarly, the compound of formula (2) is illustrated as $ClC(O)-(CH_2)_n$-$C(O)Cl$, and $(CH_2)_n$ equally is not limited to straight alkylene chains, but includes all those modifications shown above.

Accordingly, bivalent compounds of Formula I where the linkage is from a [C] group of a first ligand to a [C] group of a second ligand, i.e. a [C-C] linkage,

may be prepared from intermediates of formula (4), the preparation of which is shown below in Reaction Scheme 1.

REACTION SCHEME 1

$H_2N-(CH_2)_m-NHCO_2-t-butyl$ + $ClC(O)-(CH_2)_n-C(O)Cl$

(1)   (2)

↓

$t-butyl-OC(O)-NH-(CH_2)_m-NH-C(O)-(CH_2)_n-C(O)-NH-(CH_2)_m-NH-C(O)-t-butyl$

(3)

↓

$H_2N-(CH_2)_m-NH-C(O)-(CH_2)_n-C(O)-NH-(CH_2)_m-NH_2$ (4)
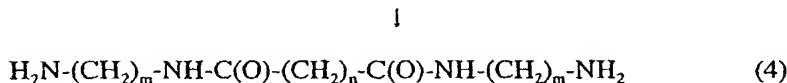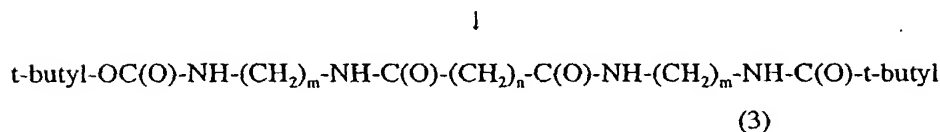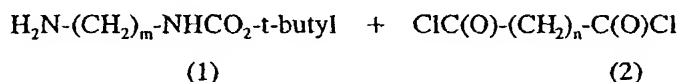
in which m and n are independently integers of 1-20.

Preparation of Compounds of Formula (3)

As illustrated in Reaction Scheme 1, step 1, about two molar equivalents of an omega-amino carbamic acid ester [formula (1)] is reacted with about one molar equivalent of a dicarboxylic acid halide, preferably chloride, of formula (2). The reaction is conducted in the presence of a non-nucleophilic base, preferably diisopropylethylamine, in an inert solvent, preferably methylene chloride, at a temperature of about 0-5°C. The mixture is then allowed to warm to room temperature. When the reaction is substantially complete, the compound of formula (3) is isolated and purified by conventional means.

Preparation of Compounds of Formula (4)

As illustrated in Reaction Scheme 1, step 2, the carbamate is removed under acid conditions. In general a preferred acid is trifluoroacetic acid, and the reaction is conducted in an inert solvent, preferably methylene chloride, at about

room temperature. When the reaction is substantially complete, the compound of formula (4) is isolated and purified by conventional means.

The compound of formula (4) is then converted into a [C-C] ligand dimer as shown in Reaction Scheme 2.

REACTION SCHEME 2

See Figure 1

Preparation of Compounds of Formula I

In general, about two molar equivalents of ligand is reacted with about one molar equivalent of the compound of formula (4), under conventional amide coupling conditions. Preferably, a hindered base is employed, preferably diisopropylethylamine, in the presence of benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) and 1-hydroybenzotriazole. The reaction is conducted in an inert polar solvent, for example, N, N-dimethylformamide (DMF) or dimethyl sulfoxide (DMSO), or preferably a mixute of both, at about room temperature. When the reaction is substantially complete, the compound of Formula I is isolated and purified by conventional means, preferably purified by reverse phase HPLC. Also isolated was a byproduct of formula (5).

Alternatively, compounds of Formula I [C-C] may be prepared from intermediates of formula (8), the preparation of which is shown below in Reaction Scheme 3.

REACTION SCHEME 3

See Figure 2

Preparation of Compounds of Formula (7)

As illustrated in Reaction Scheme 3, step 1, ligand is reacted with about 1.1 molar equivalents of a carbamic ester terminated by an alkylamino group [formula (6)]. The ester moiety is chosen for ease of removal under mild conditions in

subsequent reactions, and is preferably 9-fluorenylmethyl. Conventional amide coupling conditions are employed, preferably using PyBOP and 1-hydroxybenzotriazole. In general, the reaction is conducted in the presence of a hindered base, preferably diisopropylethylamine, in an inert polar solvent,

5   preferably DMF or DMSO, preferably a mixture of both, at about room temperature. When the reaction is substantially complete, thecompound of formula (7) is isolated and purified by conventional means.

Preparation of Compounds of Formula (8)

10   As illustrated in Reaction Scheme 3, step 2, the compound of formula (7) is reacted with a mild base to remove the protecting ester groups, which also affords decarboxylation. In general, the base is preferably piperidine, and the reaction is conducted in an inert polar solvent, preferably dimethylformamide, at about room temperature for about 10 minutes to one hour. When the reaction is substantially

15   complete, the compound of formula (8) is isolated and purified by conventional means, preferably using reverse phase HPLC.

The compound of formula (8) is then converted into a [C-C] ligand dimer as shown in Reaction Scheme 4.

20   <center>REACTION SCHEME 4</center>

<center>See Figure 3</center>

Preparation of Compounds of Formula I

As illustrated in Reaction Scheme 4, the compound of formula (8) is reacted with a dicarboxylic acid. In general, about 3 molar equivalents of the compound of

25   formula (8) is reacted with about 1 molar equivalent of the dicarboxylic acid of formula $HO_2C\text{-}(CH_2)_n\text{-}CO_2H$, under conventional amide coupling conditions. Preferably, a hindered base is employed, preferably diisopropylethlyaminine, in the presence of PyBOP and 1-hydroxybenzotriazole. The reaction is conducted in an inert polar solvent, preferably DMF, at about room temperature for about 1-3

hours. When the reaction is substantially complete, the compound of Formula I is isolated and purified by conventioanl means, preferably purified by a reverse phase of HPLC.

Compounds of Formula I wherein the linkage is [V-V] may be prepared from intermediates of formula (14), the preparation of which is shown below in Reaction Sceme 6. The starting material, the compound of formula (11), is prepared as shown in Reaction Scheme 5.

REACTION SCHEME 5

See Figure 4

Preparation of Compounds of Formula (10)

As illustrated in Reaction Scheme 5, step 1, ligand having an -NH$_2$ group suitable for linking is reacted with a protected ester-aldehyde of formula (9) to form a Schiff's base. The ester moiety is chosen for ease of removal under mild conditions in subsequent reactions, and is preferably 9-fluorenylmethyl. In general, the reaction is conducted in an inert polar solvent, preferably 3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone plus methanol, at about 50-100°C, preferably about 70°C, for about 30 minutes to 1 hour. The Schiff's base of formula (10) is not isolated, but reacted further immediately as shown below.

Preparation of Compounds of Formula (11)

As illustrated in Reaction Scheme 5, step 2, the solution of the compound of formula (10) is further reacted with a mild reducing agent. In general, the reducing agent is preferably sodium cyanoborohydride, and the reaction is conducted at about 50-100°, preferably about 70°C, for about 1-3 hours, preferably about 2 hours. When the reaction is substantially complete, the compound of formula (11) is isolated and purified by conventional means, preferably purified by reverse phase HPLC.

Compounds of Formula I wherein the linkage is [V-V] may then be prepared from intermediates of formula (11a), the preparation of which is shown below in Reaction Scheme 6.

5   REACTION SCHEME 6

See Figure 5

Preparation of Compounds of Formula (14)

As illustrated in Reaction Scheme 6, step 1, the compound of formula (11a), which is a compound of formula (11) in which the carboxyl group has been

10   protected conventionally, for example as an ester, is reacted with a mild base to remove the carbamate. In general, the base is preferably piperidine, and the reaction is conducted in an inert polar solvent, preferably DMF, at about room temperature for about 10 minutes to one hour, preferably about 30 minutes. When the reaction is substantially complete, the compound of formula (14) is isolated and

15   purified by conventional means, preferably using reverse phase HPLC.

Preparation of Compounds of Formula I

As illustrated in Reaction Scheme 6, step 2, the compound of formula (14) is reacted with a dicarboxylic acid. In general, about 3 molar equivalents of the

20   compound of formula (8) is reacted with about 1 molar equivalent of the dicarboxylic acid of formula $HO_2C-(CH_2)n-CO_2H$, under conventional amide coupling conditions. Preferably, a hindered base is employed, preferably diisopropylethylamine, in the presence of PyBOP and 1-hydroxybenzotriazole. The reaction is conducted in an inert polar solvent, preferably DMF, at bout room

25   temperature for about 1-3 hours. When the reaction is substantially complete, the protecting group R, preferably and ester, is removed conventionally, and the [V-V] compound of Formula I is isolated and purified by conventional means, preferably purified by reverse phase HPLC.

Compounds of Formula I wherein the linkage is [C-V] may be prepared from intermediates of formula (23), the preparation of which is shown below in Reaction Scheme 7. The starting material, the compound of formula (8), is prepared as previously shown.

5

REACTION SCHEME 7

See Figure 6

Preparation of Compounds of Formula (22)

As illustrated in Reaction Scheme 7, step 1, the compound of formula (8) is
10 reacted with an acid in the same manner as shown above, for example in Reaction Scheme 11, to form an amide of formula (22).

Preparation of Compounds of Formula (23)

As illustrated in Reaction Scheme 7, step 2, the compound of formula (22)
15 is hydrolyzed with an acid in the same manner as shown above, for example in Reaction Scheme 10, to form a compound of formula (23).

The compound of formula (23) is then converted into a [C-V] dimer of Formula I by reaction with a compound of formula (17), prepared as shown previously, as shown in Reaction Scheme 8.

20

REACTION SCHEME 8

See Figure 7

Preparation of Compounds of Formula I

As illustrated in Reaction Scheme 8, the compound of formula (23) is
25 reacted with a compound of formula (17) in a typical coupling reaction as shown above, to give a compound of Formula I [C-V].

Compounds of Formula I wherein linkage is [C-N] may be prepared from intermediates of formula (26), the preparation of which is shown below in Reaction Scheme 9.

REACTION SCHEME 9

See Figure 8

Preparation of Compounds of Formula (24)

5   As illustrated in Reaction Scheme 9, step 1, ligand is reacted with a
protected aminoaldehyde in the presence of an amount of base sufficient to direct
the reaction of the aldehyde to the [N] position. The Schiff's base thus formed is
reduced in the same manner as shown in Reaction Scheme 5 to form a compound of
formula (24).

10   ### Preparation of Compounds of Formula (25)

As illustrated in Reaction Scheme 9, step 2, the compound of formula (24)
is reacted with an amine in a coupling reaction in the same manner as shown above,
for example in Reaction Scheme 10, to form an amide of formula (25).

15   ### Preparation of Compounds of Formula (26)

As illustrated in Reaction Scheme 9, step 3, the protecting group FM is
removed conventionally from the compound of formula (25) with a mild base to
form a compound of formula (26).

The compound of formula (26) is then converted into a [C-N] dimer of

20   Formula I by reaction with a compound of formula (23), prepared as shown
previously, as shown in Reaction Scheme 10.

REACTION SCHEME 10

See Figure 9

25

Preparation of Compounds of Formula I

As illustrated in Reaction Scheme 10, the compound of formula (26) is
reacted with a compound of formula (23) in a typical coupling reaction as shown

above, for example in Reaction Scheme 11, to give a compound of Formula I [C-N].

Compounds of Formula I wherein the linkage is [N-V] may be prepared by a reaction of a compound of formula (26) with a compound of formula (19), as shown in Reaction Scheme 11.

REACTION SCHEME 11

See Figure 10

Preparation of Compounds of Formula I

As illustrated in Reaction Scheme 11, the compound of formula (26) is reacted with a compound of formula (19) in a typical coupling reaction as shown above, to give a compound of Formula I [N-V].

Compounds of Formula I wherein the linkage is [N-N] may be prepared by reaction of a compound formula (26) with a dicarboxylic acid of formula HO_2-C-$(CH_2)_n$-CO_2H, as shown in Reaction Scheme 12.

REACTION SCHEME 12

See Figure 11

Preparation of Compounds of Formula I

As illustrated in Reaction Scheme 12, the compound of formula (26) is reacted with a dicarboxylic acid of formula HO_2C-$(CH_2)_n$-CO_2H in the same manner as shown above in Reaction Scheme 4, to give a compound of Formula I [N-N].

Ligands that include a free hydroxy group in their structure (an alcohol or phenolic hydroxy) may be connected using those hydroxy groups as linkage points by means well known in the art. For example, one synthetic strategy that could be used for linking ligands with free hydroxy groups involves treating the ligand with t-butyl bromoacetate in the presence of a base (e.g. potassium carbonate) to convert the -OH group to an -O-CH_2CO_2-t-But group, which can be hydrolyzed to an O-CH_2CO_2H group using trifluoracetic acid. The oxtacetic group can then be used as

the linking point for two ligands by making use of the linking strategies shown above for carboxylic acids. For example, reaction of two molar equivalents of the ligand with a diamine of the formula H_2N-$(CH_2)_n$-NH_2, where n is an integer of 1-20, leads to two ligands being connected by a linker of the formula -CH_2CONH-$(CH_2)_n$-$NHCOCH_2$.

Alternatively, treating the hydroxy-bearing ligand with BOC-$NHCH_2CH_2Br$ in the presence of a base(e.g. potassium carbonate) converts the -OH group to an O-CH_2CH_2NHBOC group, which can by hydrolyzed to an O-$CH_2CH_2NH_2$ group using trifluoracetic acid. The oxyethylamino group can then be used as the linking point for two ligands by making use of the linking strategies shown above for amines. For example, reaction of two molar equivalents of the ligand with a dicarboxylic acid of the formula HO_2C-$(CH_2)_n$-CO_2H where n is an integer of 1-20, leads to two ligands being connected by a linker of the formula -$CH_2 CH_2NHCO$-$(CH_2)_n$-$CONHCH_2 CH_2$-.

Isolation and Purification of the Compounds

Isolation and purification of the compounds and intermediates described herein can be effected, if desired, by any suitable separation or purification such as, for example, filtration, extraction, crystallization, column chromatography, thin-layer chromatography, thick-layer chromatography, preparative low or high-pressure liquid chromatography or a combination of these procedures. Specific illustrations of suitable separation and isolation procedures can be had by reference to the Examples herein below. However, other equivalent separation or isolation procedures could, of course, also be used.

Combinatorial Libraries

The methods described above lend themselves to combinatorial approaches for identifying multimeric compounds which bind cellular receptors and which possess multibinding properties.

Specifically, factors such as the proper juxtaposition of the individual ligands of a multibinding compound with respect to the relevant array of binding sites on a target or targets is important in optimizing the interaction of the multibinding compound with its target(s) and to maximize the biological advantage through multivalency. One approach is to identify a library of candidate multibinding compounds with properties spanning the multibinding parameters that are relevant for a particular target. These parameters include: (1) the identity of ligand(s), (2) the orientation of ligands, (3) the valency of the construct, (4) linker length, (5) linker geometry, (6) linker physical properties, and (7) linker chemical functional groups.

Libraries of multimeric compounds potentially possessing multibinding properties (i.e., candidate multibinding compounds) and comprising a multiplicity of such variables are prepared and these libraries are then evaluated via conventional assays corresponding to the ligand selected and the multibinding parameters desired. Considerations relevant to each of these variables are set forth below:

Selection of ligand(s)

A single ligand or set of ligands is (are) selected for incorporation into the libraries of candidate multibinding compounds which library is directed against a particular biological target or targets or cellular receptors. The only requirement for the ligands chosen is that they are capable of interacting with the selected target(s). Thus, ligands may be known drugs, modified forms of known drugs, substructures of known drugs or substrates of modified forms of known drugs (which are competent to interact with the target), or other compounds. Ligands are preferably chosen based on known favorable properties that may be projected to be carried over to or amplified in multibinding forms. Favorable properties include demonstrated safety and efficacy in human patients, appropriate PK/ADME profiles, synthetic accessibility, and desirable physical properties such as solubility,

logP, etc. However, it is crucial to note that ligands which display an unfavorable property from among the previous list may obtain a more favorable property through the process of multibinding compound formation; i.e., ligands should not necessarily be excluded on such a basis. For example, a ligand that is not

5   sufficiently potent at a particular target so as to be efficacious in a human patient may become highly potent and efficacious when presented in multibinding form. A ligand that is potent and efficacious but not of utility because of a non-mechanism-related toxic side effect may have increased therapeutic index (increased potency relative to toxicity) as a multibinding compound. Compounds that exhibit short *in*

10   *vivo* half-lives may have extended half-lives as multibinding compounds. Physical properties of ligands that limit their usefulness (e.g. poor bioavailability due to low solubility, hydrophobicity, hydrophilicity) may be rationally modulated in multibinding forms, providing compounds with physical properties consistent with the desired utility.

15

Orientation: selection of ligand attachment points and linking chemistry

         Several points are chosen on each ligand at which to attach the ligand to the linker. The selected points on the ligand/linker for attachment are functionalized to contain complementary reactive functional groups. This permits probing the

20   effects of presenting the ligands to their receptor(s) in multiple relative orientations, an important multibinding design parameter. The only requirement for choosing attachment points is that attaching to at least one of these points does not abrogate activity of the ligand. Such points for attachment can be identified by structural information when available. For example, inspection of a co-crystal structure of a

25   protease inhibitor bound to its target allows one to identify one or more sites where linker attachment will not preclude the enzyme:inhibitor interaction. Alternatively, evaluation of ligand/target binding by nuclear magnetic resonance will permit the identification of sites non-essential for ligand/target binding. See, for example, Fesik, et al., U.S. Patent No. 5,891,643. When such structural information is not

available, utilization of structure-activity relationships (SAR) for ligands will suggest positions where substantial structural variations are and are not allowed. In the absence of both structural and SAR information, a library is merely selected with multiple points of attachment to allow presentation of the ligand in multiple

5   distinct orientations. Subsequent evaluation of this library will indicate what positions are suitable for attachment.

It is important to emphasize that positions of attachment that do abrogate the activity of the monomeric ligand may also be advantageously included in candidate multibinding compounds in the library provided that such compounds bear at least

10   one ligand attached in a manner which does not abrogate intrinsic activity. This selection derives from, for example, heterobivalent interactions within the context of a single target molecule. For example, consider a receptor antagonist ligand bound to its target receptor, and then consider modifying this ligand by attaching to it a second copy of the same ligand with a linker which allows the second ligand to

15   interact with the same receptor molecule at sites proximal to the antagonist binding site, which include elements of the receptor that are not part of the formal antagonist binding site and/or elements of the matrix surrounding the receptor such as the membrane. Here, the most favorable orientation for interaction of the second ligand molecule with the receptor/matrix may be achieved by attaching it to

20   the linker at a position which abrogates activity of the ligand at the formal antagonist binding site. Another way to consider this is that the SAR of individual ligands within the context of a multibinding structure is often different from the SAR of those same ligands in momomeric form.

The foregoing discussion focused on bivalent interactions of dimeric

25   compounds bearing two copies of the same ligand joined to a single linker through different attachment points, one of which may abrogate the binding/activity of the monomeric ligand. It should also be understood that bivalent advantage may also be attained with heterodimeric constructs bearing two different ligands that bind to common or different targets. For example, a 5HT$_4$ receptor antagonist and a

bladder-selective muscarinic M_3 antagonist may be joined to a linker through attachment points which do not abrogate the binding affinity of the monomeric ligands for their respective receptor sites. The dimeric compound may achieve enhanced affinity for both receptors due to favorable interactions between the $5HT_4$ ligand and elements of the M_3 receptor proximal to the formal M_3 antagonist binding site and between the M_3 ligand and elements of the $5HT_4$ receptor proximal to the formal $5HT_4$ antagonist binding site. Thus, the dimeric compound may be more potent and selective antagonist of overactive bladder and a superior therapy for urinary urge incontinence.

Once the ligand attachment points have been chosen, one identifies the types of chemical linkages that are possible at those points. The most preferred types of chemical linkages are those that are compatible with the overall structure of the ligand (or protected forms of the ligand) readily and generally formed, stable and intrinsically inocuous under typical chemical and physiological conditions, and compatible with a large number of available linkers. Amide bonds, ethers, amines, carbamates, ureas, and sulfonamides are but a few examples of preferred linkages.

Linkers: spanning relevant multibinding parameters through selection of valency, linker length, linker geometry, rigidity, physical properties, and chemical functional groups

In the library of linkers employed to generate the library of candidate multibinding compounds, the selection of linkers employed in this library of linkers takes into consideration the following factors:

Valency. In most instances the library of linkers is initiated with divalent linkers. The choice of ligands and proper juxtaposition of two ligands relative to their binding sites permits such molecules to exhibit target binding affinities and specificities more than sufficient to confer biological advantage. Furthermore,

divalent linkers or constructs are also typically of modest size such that they retain the desirable biodistribution properties of small molecules.

Linker length. Linkers are chosen in a range of lengths to allow the
5   spanning of a range of inter-ligand distances that encompass the distance preferable for a given divalent interaction. In some instances the preferred distance can be estimated rather precisely from high-resolution structural information of targets, typically enzymes and soluble receptor targets. In other instances where high-resolution structural information is not available (such as 7TM G-protein coupled
10   receptors), one can make use of simple models to estimate the maximum distance between binding sites either on adjacent receptors or at different locations on the same receptor. In situations where two binding sites are present on the same target (or target subunit for multisubunit targets), preferred linker distances are 2-20 Å, with more preferred linker distances of 3-12 Å. In situations where two binding
15   sites reside on separate (e.g., protein) target sites, preferred linker distances are 20-100 Å, with more preferred distances of 30-70 Å.

Linker geometry and rigidity. The combination of ligand attachment site, linker length, linker geometry, and linker rigidity determine the possible ways in
20   which the ligands of candidate multibinding compounds may be displayed in three dimensions and thereby presented to their binding sites. Linker geometry and rigidity are nominally determined by chemical composition and bonding pattern, which may be controlled and are systematically varied as another spanning function in a multibinding array. For example, linker geometry is varied by attaching two
25   ligands to the ortho, meta, and para positions of a benzene ring, or in *cis-* or *trans-*arrangements at the 1,1- vs. 1,2- vs. 1,3- vs. 1,4- positions around a cyclohexane core or in *cis-* or *trans-*arrangements at a point of ethylene unsaturation. Linker rigidity is varied by controlling the number and relative energies of different conformational states possible for the linker. For example, a divalent compound

bearing two ligands joined by 1,8-octyl linker has many more degrees of freedom, and is therefore less rigid than a compound in which the two ligands are attached to the 4,4' positions of a biphenyl linker.

5   Linker physical properties. The physical properties of linkers are nominally determined by the chemical constitution and bonding patterns of the linker, and linker physical properties impact the overall physical properties of the candidate multibinding compounds in which they are included. A range of linker compositions is typically selected to provide a range of physical properties

10   (hydrophobicity, hydrophilicity, amphiphilicity, polarization, polarizability, acidity, and basicity) in the candidate multibinding compounds. The particular choice of linker physical properties is made within the context of the physical properties of the ligands they join and preferably the goal is to generate molecules with favorable PK/ADME properties. For example, linkers can be selected to

15   avoid those that are too hydrophilic or too hydrophobic to be readily absorbed and/or distributed *in vivo*.

           Linker chemical functional groups. Linker chemical functional groups are selected to be compatible with the chemistry chosen to connect linkers to the

20   ligands and to impart the range of physical properties sufficient to span initial examination of this parameter.

Combinatorial synthesis

           Having chosen a set of n ligands (n being determined by the sum of the

25   number of different attachment points for each ligand chosen) and m linkers by the process outlined above, a library of $(n!)m$ candidate divalent multibinding compounds is prepared which spans the relevant multibinding design parameters for a particular target. For example, an array generated from two ligands, one which has two attachment points (A1, A2) and one which has three attachment

points (B1, B2, B3) joined in all possible combinations provide for at least 15 possible combinations of multibinding compounds:

A1-A1	A1-A2	A1-B1	A1-B2	A1-B3	A2-A2	A2-B1	A2-B2
A2-B3	B1-B1	B1-B2	B1-B3	B2-B2	B2-B3	B3-B3	

When each of these combinations is joined by 10 different linkers, a library of 150 candidate multibinding compounds results.

Given the combinatorial nature of the library, common chemistries are preferably used to join the reactive functionalies on the ligands with complementary reactive functionalities on the linkers. The library therefore lends itself to efficient parallel synthetic methods. The combinatorial library can employ solid phase chemistries well known in the art wherein the ligand and/or linker is attached to a solid support. Alternatively and preferably, the combinatorial libary is prepared in the solution phase. After synthesis, candidate multibinding compounds are optionally purified before assaying for activity by, for example, chromatographic methods (e.g., HPLC).

Analysis of array by biochemical, analytical, pharmacological, and computational methods

Various methods are used to characterize the properties and activities of the candidate multibinding compounds in the library to determine which compounds possess multibinding properties. Physical constants such as solubility under various solvent conditions and logD/clogD values can be determined. A combination of NMR spectroscopy and computational methods is used to determine low-energy conformations of the candidate multibinding compounds in fluid media. The ability of the members of the library to bind to the desired target and other targets is determined by various standard methods, which include radioligand displacement assays for receptor and ion channel targets, and kinetic inhibition

analysis for many enzyme targets. *In vitro* efficacy, such as for receptor agonists and antagonists, can also be determined. Pharmacological data, including oral absorption, everted gut penetration, other pharmacokinetic parameters and efficacy data can be determined in appropriate models. In this way, key structure-activity relationships are obtained for multibinding design parameters which are then used to direct future work.

The members of the library which exhibit multibinding properties, as defined herein, can be readily determined by conventional methods. First those members which exhibit multibinding properties are identified by conventional methods as described above including conventional assays (both *in vitro* and *in vivo*).

Second, ascertaining the structure of those compounds which exhibit multibinding properties can be accomplished via art recognized procedures. For example, each member of the library can be encrypted or tagged with appropriate information allowing determination of the structure of relevant members at a later time. See, for example, Dower, et al., International Patent Application Publication No. WO 93/06121; Brenner, et al., Proc. Natl. Acad. Sci., USA, 89:5181 (1992); Gallop, et al., U.S. Patent No. 5,846,839; each of which are incorporated herein by reference in its entirety. Alternatively, the structure of relevant multivalent compounds can also be determined from soluble and untagged libaries of candidate multivalent compounds by methods known in the art such as those described by Hindsgaul, et al., Canadian Patent Application No. 2,240,325 which was published on July 11, 1998. Such methods couple frontal affinity chromatography with mass spectroscopy to determine both the structure and relative binding affinities of candidate multibinding compounds to receptors.

The process set forth above for dimeric candidate multibinding compounds can, of course, be extended to trimeric candidate compounds and higher analogs thereof.

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Follow-up synthesis and analysis of additional array(s)

Based on the information obtained through analysis of the initial library, an optional component of the process is to ascertain one or more promising multibinding "lead" compounds as defined by particular relative ligand

5   orientations, linker lengths, linker geometries, etc. Additional libraries can then be generated around these leads to provide for further information regarding structure to activity relationships. These arrays typically bear more focused variations in linker structure in an effort to further optimize target affinity and/or activity at the target (antagonism, partial agonism, etc.), and/or alter physical properties. By

10   iterative redesign/analysis using the novel principles of multibinding design along with classical medicinal chemistry, biochemistry, and pharmacology approaches, one is able to prepare and identify optimal multibinding compounds that exhibit biological advantage towards their targets and as therapeutic agents.

To further elaborate upon this procedure, suitable divalent linkers include,

15   by way of example only, those derived from dicarboxylic acids, disulfonylhalides, dialdehydes, diketones, dihalides, diisocyanates,diamines, diols, mixtures of carboxylic acids, sulfonylhalides, aldehydes, ketones, halides, isocyanates, amines and diols. In each case, the carboxylic acid, sulfonylhalide, aldehyde, ketone, halide, isocyanate, amine and diol functional group is reacted with a

20   complementary functionality on the ligand to form a covalent linkage. Such complementary functionality is well known in the art as illustrated in the following table:

COMPLEMENTARY BINDING CHEMISTRIES

25

First Reactive Group	Second Reactive Group	Linkage
hydroxyl	isocyanate	urethane
amine	epoxide	β-hydroxyamine
sulfonyl halide	amine	sulfonamide

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carboxyl acid	amine	amide
hydroxyl	alkyl/aryl halide	ether
aldehyde	amine/NaCNBH$_4$	amine
ketone	amine/NaCNBH$_4$	amine
amine	isocyanate	urea

Exemplary linkers include the following linkers identified as X-1 through X-418 as set forth below:

-110a-

Diacids

X-1

X-2

X-3

X-4

X-5

X-6

X-7

X-8

X-9

X-10

X-11

X-12

X-13

X-14

X-15

X-16

X-17

X-18

X-19

-110 b-

X-20

X-21

X-22

X-23

X-24

X-25

X-26

Chiral
X-27

X-28

X-29

X-30

X-31

Chiral
X-32

-110c-

X-33
Chiral

X-34
Chiral

X-35

X-36

X-37

X-38

X-39

X-40

X-41
Chiral

X-42

X-43

X-44

X-45

X-46
Chiral

X-47

X-48

-110d-

X-49

X-50
Chiral

X-51

X-52
Chiral

X-53
Chiral

X-54
Chiral

X-55

X-56

X-57

X-58
Chiral

X-59
Chiral

X-60

X-61

X-62
Chiral

X-63

Chiral
X-64

X-65

X-66

X-67

X-68

X-69

Chiral
X-70

Chiral
X-71

X-72

X-73

X-74

X-75

X-76

X-77

X-78

X-79

-111a-

H₃C—
H₃C— O   N   O   N   OH
   CH₃   OH
   O
Chiral
X-80

Benzyl O   N   O   N   O   OH
              CH₃   OH
                    O   OH
Chiral
X-81

Benzyl O   N
HO   N   O   OH
   O   O
Chiral
X-82

O   OH
HO   O
   HO   OH
X-83

O   OH
HO   O
X-84

-III b-

X-85

Chiral
X-86

X-87

X-88

X-89

X-90

X-91

Chiral
X-92

X-93

X-94

X-95

X-96

X-97

-IIIc-

X-98

X-99

X-100

X-101

X-102

X-103

X-104

X-105

X-106

X-107

X-108

X'-109

–IIId–

X–110

Chiral
X–110

X–111

Chiral
X–112

X–113

X–114

Chiral
X–115

Chiral
X–116

X–117

X–118

X–119

X–120

X–121

X–122

Chiral
X–123

X–124

X–125

X–126

Chiral
X-127

X-128

X-129

X-130

X-131

X-132

Disulfonyl Halides

X-133

X-134

X-135

X-136

X-137

X-138

X-139

X-140

X-141

– 111f –

X–142

X–143

X–144

X–145

X–146

X–147

X–148

X–149

X–150

X–151

X–152

Dialdehydes

X–153

X–154

X–155

X–156

X–157

X–158

X-159

X-160

X-161

X-162

X-163

X-164

X-165

X-166

X-167

X-168

X-169

X-170

X-171

X-172

X-173

X-174

X-176

X-177

Dihalides

X-175

X-178

X-179

X-180

-112 b-

Br—⟋⟍⟋⟍⟋⟍⟋⟍⟋—Br

X-181

Cl—⟍⟋O—C(=O)—O⟍⟋—Cl

X-182

X-183

I—⟍⟋⟍⟋—I

X-184

Br—⟍⟋—Br

X-185

Cl—⟍C(=CH₂)⟋—Cl

X-186

Br—⟋⟍⟋⟍⟋⟍⟋⟍⟋—Br

X-187

X-188

X-189

X-190

X-191

I—⟍⟋⟍—I

X-192

Br—⟋⟍⟋⟍⟋⟍⟋⟍⟋⟍—Br

X-193

X-194

X-195

Cl—⟍⟋—N(CH₃)(ClH)—⟍⟋—Cl

X-196

HO—CH₂—C(CH₂Br)(CH₂OH)—...

X-197

F₃C—CHBr—CH₂Br

X-198

Br—⟋⟍⟋⟍⟋—Br

X-199

I—⟍⟋⟍⟋⟍—I

X-200

X-201

X-202

Br—⟍⟋(Br)⟋—OH

X-203

X-204

Br—⟋⟍⟋⟍⟋⟍—Br

X-205

Br—⟍⟋—O—⟍⟋—Br

X-206

H₃C—O—C(CH₂Br)(CH₂Br)—O—CH₃

X-207

H₂N—C(=O)—CHBr—CH₂Br

X-208

Cl—CH₂—(C₆H₄)—CH₂—Cl

X-209

Br—⟍⟋=⟋⟍—Br

X-210

Br—⟋⟍⟋⟍⟋⟍⟋⟍—Br

X-211

Br—⟍⟋CH(CH₃)⟋⟍—Br

X-212

X-213

X-214

Diisocyanates

X-215

X-216

X-217

X-218

X-219

X-220

X-221

X-222

X-223

X-224

X-225

X-226

X-227

X-228

X-229

X-230

X-231

X-232

-113b-

X-233

X-234

X-235

X-236

X-237

X-238

X-239

X-240

X-241

X-242

X-243

X-244

X-245

– 113 c –

X–246

X–247

X–248

Diamines

X–249

X–250

X–251

X–252

X–253

X–254

X–256

X–255

X–257

X–258

X–259

X–260

X–261

X–262

X–263

X–264

X–265

X-266

X-267

X-268

X-269

X-270

X-271

X-272

X-273

X-274

X-275

X-277

X-276

X-278

X-279

X-280

X-281

X-282

X-283

-113e-

H2N–\/\–O–\/–O–\/–O–\/\–NH2
X-285

H3C–\/\–N–\/\/\–N–\/\–CH3
X-286

H2N–\/\/\/–NH2
X-287

X-288 (diaminodiphenyl sulfone, meta)

H2N–\/–N–CH3
X-289

H2N–\/–C(CH3)(CH2NH2) with H3C, CH3
X-290

X-292 (N,N'-dibenzyl ethylenediamine)

H2N–\/\–O–\/\/\–O–\/\–NH2
X-291

H2N–\/\/\/\–NH2
X-293

X-294 (4,4'-diaminodiphenyl sulfone)

X-295 (cyclohexane dimethanamine)

X-296 (diaminodiphenylmethane, meta)

X-297

H3C–\/–N–\/\/–N–\/–CH3
X-298

H2N–\/\/\/\/–NH2
X-299

X-300

X-301

X-302

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X-303

X-304

X-305

X-306
Chiral

X-307

X-308

X-309

X-310

X-311

X-312
Chiral

X-313

X-314

-114b-

H_2N — (chain) — NH_2

X-315

H_3C / CH_3 CH, H_3C—N—(chain)—N, H_3C / CH_3

X-316

Cl, N—CH₂—(cyclohexane)—CH₂—N, Cl, Cl, Cl

X-317

H_3C—N, (chiral diphenyl) N—CH_3

Chiral
X-318

H_2N—(benzene)—CH_2—NH_2

X-319

H_2N, H_3C / CH_3 C NH_2

X-320

H_3C—N—(chain)—N—CH_3

X-321

H_2N—(chain)—NH_2

X-322

H_3C—N—(alkene)—N—CH_3

X-323

H_3C—N—(chain)—N—CH_3

X-324

H_2N—(ether)—O—(ether)—NH_2

X-325

Diols

HO—(chain)—O—(bromophenyl)—C(CH₃)(H₃C)—(bromophenyl)—O—(chain)—OH

Br, H₃C, CH₃, Br, Br, Br

X-326

HO—(chain)—O—(chain)—O—(chain)—OH

X-327

HO—(chain)—N—C(=O)—C(=O)—N—(chain)—OH

X-328

(piperidine)—N—CH₂CH₂—OH, (piperidine)—N—CH₂CH₂—OH

X-329

X-330

X-331

X-332

X-333

X-334

X-335

X-336

X-337

X-338

X-339

X-340

X-341

X-342

X-343

X-344

-114d-

HO–CH₂CH₂–S–CH₂CH₂–S–CH₂CH₂–OH

X-345

HO–CH₂ [tricyclic] CH₂–OH

X-346

HO–CH₂CH₂–S(=O)(=O)–CH₂CH₂–OH

X-347

H₃C–CH(OH)–CH₂CH₂–CH(OH)–CH₃

X-348

HO–CH₂CH₂CH₂–OH

X-349

HO–CH₂CH₂–O–CH₂CH₂–O–CH₂CH₂–O–CH₂CH₂–OH

X-350

HO–CH₂CH₂–O–[C₆H₄]–O–CH₂CH₂–OH

X-351

HO–(CH₂)₁₀–OH

X-352

HO–CH₂–[C₆H₄]–CH₂–OH

X-353

X-354

X-355

X-356

HO–(CH₂)₆–OH

X-357

HO–(CH₂)₇–OH

X-358

H₃C–CH(OH)–CH₂CH₂CH₂–OH

X-359

X-360

-114e-

X-361

X-362

X-363

X-364

X-365

X-366

X-367

X-368

X-369

X-370

X-371

X-372

X-373

X-374

X-375

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HO ~~~~~~ OH

X-376

H_3C, HO — (with benzene ring, $C(CH_3)_2OH$) substituents

X-377

HO ~~~ OH

X-378

HO~N(CH$_2$CH$_3$, CH$_3$)~OH

X-379

HO~O~C≡C~O~OH

X-380

HO~S-S~OH

X-381

HO~O~O~OH

X-382

HO~O~OH

X-383

HO~C≡C~OH

X-384

HO~CF$_2$CF$_2$~OH (fluorinated)

X-385

Dithiols

HS ~~~~~~ SH

X-386

HS ~~~~~ SH

X-387

HS—(benzene ring with Cl)—SH

X-388

HS—(benzene ring with CH$_3$)—(HS)

X-389

SH—(benzene ring)—SH

X-390

HS ~~~ SH

X-391

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HS—CH2—C(=O)—O—CH2CH2—O—C(=O)—CH2—SH
X-392

HS—(CH2)10—SH
X-393

benzene-1,2-dithiol (HS, HS)
X-394

HS—CH2—CH(SH)—CH2—OH
X-395

tetramethylbenzene dithiol (SH, CH3, SH, H3C, CH3)
X-396

naphthalene-1,5-dithiol (SH, SH)
X-397

HS—CH2CH2—O—CH2CH2—O—CH2CH2—SH
X-398

HS, HS—...—C(=O)—OH
X-399

benzene-1,3-dithiol (HS, SH)
X-400

dimethylbenzene dithiol (CH3, CH3, HS, SH)
X-401

HS—CH2CH2—S(=O)2—CH2CH2—SH
X-402

HS—CH2CH2CH2—SH
X-403

HS—(CH2)10—SH
X-404

HS—CH2CH2—C(=O)—N—C(=O)—CH2CH2—SH
X-405

p-xylylene dithiol (HS—CH2, CH2—SH)
X-406

HS—CH2CH2—S—CH2CH2—SH
X-407

HS—(CH2)5—SH
X-408

HS—(CH2)4—SH
X-409

HS—CH2—CH(OH)—CH(OH)—CH2—SH
X-410

HO2C—CH(SH)—CH2—CH(SH)—CO2H
X-411

HS—CH2—CH(OH)—CH(OH)—CH2—SH
X-412

HS—CH2CH2—O—CH2CH2—SH
X-413

o-xylylene dithiol (HS—CH2, CH2—SH)
X-414

HO2C—CH(SH)—CH(SH)—CO2H
X-415

Chiral HS—CH2—CH(OH)—CH(OH)—CH2—SH
X-416

bis(4-mercaptophenyl) sulfide (HS, S, SH)
X-417

m-xylylene dithiol (HS—CH2, CH2—SH)
X-418

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Representative ligands for use in this invention include, by way of example, L as identified above (also identified as L-1).

Combinations of ligands (L) and linkers (X) per this invention include, by way example only, homo- and hetero-dimers wherein a first ligand is selected from L-1 above and the second ligand and linker is selected from the following:

L-1/X-1-	L-1/X-2-	L-1/X-3-	L-1/X-4-	L-1/X-5-	L-1/X-6-
L-1/X-7-	L-1/X-8-	L-1/X-9-	L-1/X-10-	L-1/X-11-	L-1/X-12-
L-1/X-13-	L-1/X-14-	L-1/X-15-	L-1/X-16-	L-1/X-17-	L-1/X-18-
L-1/X-19-	L-1/X-20-	L-1/X-21-	L-1/X-22-	L-1/X-23-	L-1/X-24-
L-1/X-25-	L-1/X-26-	L-1/X-27-	L-1/X-28-	L-1/X-29-	L-1/X-30-
L-1/X-31-	L-1/X-32-	L-1/X-33-	L-1/X-34-	L-1/X-35-	L-1/X-36-
L-1/X-37-	L-1/X-38-	L-1/X-39-	L-1/X-40-	L-1/X-41-	L-1/X-42-
L-1/X-43-	L-1/X-44-	L-1/X-45-	L-1/X-46-	L-1/X-47-	L-1/X-48-
L-1/X-49-	L-1/X-50-	L-1/X-51-	L-1/X-52-	L-1/X-53-	L-1/X-54-
L-1/X-55-	L-1/X-56-	L-1/X-57-	L-1/X-58-	L-1/X-59-	L-1/X-60-
L-1/X-61-	L-1/X-62-	L-1/X-63-	L-1/X-64-	L-1/X-65-	L-1/X-66-
L-1/X-67-	L-1/X-68-	L-1/X-69-	L-1/X-70-	L-1/X-71-	L-1/X-72-
L-1/X-73-	L-1/X-74-	L-1/X-75-	L-1/X-76-	L-1/X-77-	L-1/X-78-
L-1/X-79-	L-1/X-80-	L-1/X-81-	L-1/X-82-	L-1/X-83-	L-1/X-84-
L-1/X-85-	L-1/X-86-	L-1/X-87-	L-1/X-88-	L-1/X-89-	L-1/X-90-
L-1/X-91-	L-1/X-92-	L-1/X-93-	L-1/X-94-	L-1/X-95-	L-1/X-96-
L-1/X-97-	L-1/X-98-	L-1/X-99-	L-1/X-100-	L-1/X-101-	L-1/X-102-
L-1/X-103-	L-1/X-104-	L-1/X-105-	L-1/X-106-	L-1/X-107-	L-1/X-108-
L-1/X-109-	L-1/X-110-	L-1/X-111-	L-1/X-112-	L-1/X-113-	L-1/X-114-
L-1/X-115-	L-1/X-116-	L-1/X-117-	L-1/X-118-	L-1/X-119-	L-1/X-120-
L-1/X-121-	L-1/X-122-	L-1/X-123-	L-1/X-124-	L-1/X-125-	L-1/X-126-
L-1/X-127-	L-1/X-128-	L-1/X-129-	L-1/X-130-	L-1/X-131-	L-1/X-132-
L-1/X-133-	L-1/X-134-	L-1/X-135-	L-1/X-136-	L-1/X-137-	L-1/X-138-
L-1/X-139-	L-1/X-140-	L-1/X-141-	L-1/X-142-	L-1/X-143-	L-1/X-144-
L-1/X-145-	L-1/X-146-	L-1/X-147-	L-1/X-148-	L-1/X-149-	L-1/X-150-

L-1/X-151-	L-1/X-152-	L-1/X-153-	L-1/X-154-	L-1/X-155-	L-1/X-156-
L-1/X-157-	L-1/X-158-	L-1/X-159-	L-1/X-160-	L-1/X-161-	L-1/X-162-
L-1/X-163-	L-1/X-164-	L-1/X-165-	L-1/X-166-	L-1/X-167-	L-1/X-168-
L-1/X-169-	L-1/X-170-	L-1/X-173-	L-1/X-172-	L-1/X-173-	L-1/X-174-
L-1/X-175-	L-1/X-176-	L-1/X-177-	L-1/X-178-	L-1/X-179-	L-1/X-180-
L-1/X-181-	L-1/X-182-	L-1/X-183-	L-1/X-184-	L-1/X-185-	L-1/X-186-
L-1/X-187-	L-1/X-188-	L-1/X-189-	L-1/X-190-	L-1/X-191-	L-1/X-192-
L-1/X-193-	L-1/X-194-	L-1/X-195-	L-1/X-196-	L-1/X-197-	L-1/X-198-
L-1/X-199-	L-1/X-200-	L-1/X-201-	L-1/X-202-	L-1/X-203-	L-1/X-204-
L-1/X-205-	L-1/X-206-	L-1/X-207-	L-1/X-208-	L-1/X-209-	L-1/X-210-
L-1/X-211-	L-1/X-212-	L-1/X-213-	L-1/X-214-	L-1/X-215-	L-1/X-216-
L-1/X-217-	L-1/X-218-	L-1/X-219-	L-1/X-220-	L-1/X-221-	L-1/X-222-
L-1/X-223-	L-1/X-224-	L-1/X-225-	L-1/X-226-	L-1/X-227-	L-1/X-228-
L-1/X-229-	L-1/X-230-	L-1/X-231-	L-1/X-232-	L-1/X-233-	L-1/X-234-
L-1/X-235-	L-1/X-236-	L-1/X-237-	L-1/X-238-	L-1/X-239-	L-1/X-240-
L-1/X-241-	L-1/X-242-	L-1/X-243-	L-1/X-244-	L-1/X-245-	L-1/X-246-
L-1/X-247-	L-1/X-248-	L-1/X-249-	L-1/X-250-	L-1/X-251-	L-1/X-252-
L-1/X-253-	L-1/X-254-	L-1/X-255-	L-1/X-256-	L-1/X-257-	L-1/X-258-
L-1/X-259-	L-1/X-260-	L-1/X-261-	L-1/X-262-	L-1/X-263-	L-1/X-264-
L-1/X-265-	L-1/X-266-	L-1/X-267-	L-1/X-268-	L-1/X-269-	L-1/X-270-
L-1/X-271-	L-1/X-272-	L-1/X-273-	L-1/X-274-	L-1/X-275-	L-1/X-276-
L-1/X-277-	L-1/X-278-	L-1/X-279-	L-1/X-280-	L-1/X-281-	L-1/X-282-
L-1/X-283-	L-1/X-284-	L-1/X-285-	L-1/X-286-	L-1/X-287-	L-1/X-288-
L-1/X-289-	L-1/X-290-	L-1/X-291-	L-1/X-292-	L-1/X-293-	L-1/X-294-
L-1/X-295-	L-1/X-296-	L-1/X-297-	L-1/X-298-	L-1/X-299-	L-1/X-300-
L-1/X-301-	L-1/X-302-	L-1/X-303-	L-1/X-304-	L-1/X-305-	L-1/X-306-
L-1/X-307-	L-1/X-308-	L-1/X-309-	L-1/X-310-	L-1/X-311-	L-1/X-312-
L-1/X-313-	L-1/X-314-	L-1/X-315-	L-1/X-316-	L-1/X-317-	L-1/X-318-
L-1/X-319-	L-1/X-320-	L-1/X-321-	L-1/X-322-	L-1/X-323-	L-1/X-324-
L-1/X-325-	L-1/X-326-	L-1/X-327-	L-1/X-328-	L-1/X-329-	L-1/X-330-
L-1/X-331-	L-1/X-332-	L-1/X-333-	L-1/X-334-	L-1/X-335-	L-1/X-336-
L-1/X-337-	L-1/X-338-	L-1/X-339-	L-1/X-340-	L-1/X-341-	L-1/X-342-

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L-1/X-343-	L-1/X-344-	L-1/X-345-	L-1/X-346-	L-1/X-347-	L-1/X-348-
L-1/X-349-	L-1/X-350-	L-1/X-351-	L-1/X-352-	L-1/X-353-	L-1/X-354-
L-1/X-355-	L-1/X-356-	L-1/X-357-	L-1/X-358-	L-1/X-359-	L-1/X-360-
L-1/X-361-	L-1/X-362-	L-1/X-363-	L-1/X-364-	L-1/X-365-	L-1/X-366-
L-1/X-367-	L-1/X-368-	L-1/X-369-	L-1/X-370-	L-1/X-371-	L-1/X-372-
L-1/X-373-	L-1/X-374-	L-1/X-375-	L-1/X-376-	L-1/X-377-	L-1/X-378-
L-1/X-379-	L-1/X-380-	L-1/X-381-	L-1/X-382-	L-1/X-383-	L-1/X-384-
L-1/X-385-	L-1/X-386-	L-1/X-387-	L-1/X-388-	L-1/X-389-	L-1/X-390-
L-1/X-391-	L-1/X-392-	L-1/X-393-	L-1/X-394-	L-1/X-395-	L-1/X-396-
L-1/X-397-	L-1/X-398-	L-1/X-399-	L-1/X-400-	L-1/X-401-	L-1/X-402-
L-1/X-403-	L-1/X-404-	L-1/X-405-	L-1/X-406-	L-1/X-407-	L-1/X-408-
L-1/X-409-	L-1/X-410-	L-1/X-411-	L-1/X-412-	L-1/X-413-	L-1/X-414-
L-1/X-415-	L-1/X-416-	L-1/X-417-	L-1/X-418-		

Pharmaceutical Formulations

When employed as pharmaceuticals, the compounds of formula I are usually administered in the form of pharmaceutical compositions. These compounds can be administered by a variety of routes including oral, rectal, transdermal, subcutaneous, intravenous, intramuscular, and intranasal. These compounds are effective as both injectable and oral compositions. Such compositions are prepared in a manner well known in the pharmaceutical art and comprise at least one active compound.

This invention also includes pharmaceutical compositions which contain, as the active ingredient, one or more of the compounds of formula I above associated with pharmaceutically acceptable carriers. In making the compositions of this invention, the active ingredient is usually mixed with an excipient, diluted by an excipient or enclosed within such a carrier which can be in the form of a capsule, sachet, paper or other container. When the excipient serves as a diluent, it can be a solid, semi-solid, or liquid material, which acts as a vehicle, carrier or medium for the active ingredient. Thus, the compositions can be in the form of tablets, pills,

powders, lozenges, sachets, cachets, elixirs, suspensions, emulsions, solutions, syrups, aerosols (as a solid or in a liquid medium), ointments containing, for example, up to 10% by weight of the active compound, soft and hard gelatin capsules, suppositories, sterile injectable solutions, and sterile packaged powders.

5   In preparing a formulation, it may be necessary to mill the active compound to provide the appropriate particle size prior to combining with the other ingredients. If the active compound is substantially insoluble, it ordinarily is milled to a particle size of less than 200 mesh. If the active compound is substantially water soluble, the particle size is normally adjusted by milling to

10   provide a substantially uniform distribution in the formulation, e.g. about 40 mesh.

Some examples of suitable excipients include lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, calcium phosphate, alginates, tragacanth, gelatin, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, sterile water, syrup, and methyl cellulose. The formulations can

15   additionally include: lubricating agents such as talc, magnesium stearate, and mineral oil; wetting agents; emulsifying and suspending agents; preserving agents such as methyl- and propylhydroxy-benzoates; sweetening agents; and flavoring agents. The compositions of the invention can be formulated so as to provide quick, sustained or delayed release of the active ingredient after administration to

20   the patient by employing procedures known in the art.

The compositions are preferably formulated in a unit dosage form, each dosage containing from about 5 to about 100 mg, more usually about 10 to about 30 mg, of the active ingredient. The term "unit dosage forms" refers to physically discrete units suitable as unitary dosages for human subjects and other mammals,

25   each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect, in association with a suitable pharmaceutical excipient. Preferably, the compound of formula I above is employed at no more than about 20 weight percent of the pharmaceutical composition, more preferably

no more than about 15 weight percent, with the balance being pharmaceutically inert carrier(s).

The active compound is effective over a wide dosage range and is generally administered in a pharmaceutically effective amount. It, will be understood,
5   however, that the amount of the compound actually administered will be determined by a physician or veterinarian, in the light of the relevant circumstances, including the condition to be treated, the chosen route of administration, the actual compound administered and its relative activity, the age, weight, and response of the individual patient, the severity of the patient's
10 symptoms, and the like.

For preparing solid compositions such as tablets, the principal active ingredient is mixed with a pharmaceutical excipient to form a solid preformulation composition containing a homogeneous mixture of a compound of the present invention. When referring to these preformulation compositions as homogeneous,
15 it is meant that the active ingredient is dispersed evenly throughout the composition so that the composition may be readily subdivided into equally effective unit dosage forms such as tablets, pills and capsules. This solid preformulation is then subdivided into unit dosage forms of the type described above containing from, for example, 0.1 to about 500 mg of the active ingredient of the present invention.

20 The tablets or pills of the present invention may be coated or otherwise compounded to provide a dosage form affording the advantage of prolonged action. For example, the tablet or pill can comprise an inner dosage and an outer dosage component, the latter being in the form of an envelope over the former. The two components can be separated by an enteric layer which serves to resist
25 disintegration in the stomach and permit the inner component to pass intact into the duodenum or to be delayed in release. A variety of materials can be used for such enteric layers or coatings, such materials including a number of polymeric acids and mixtures of polymeric acids with such materials as shellac, cetyl alcohol, and cellulose acetate.

The liquid forms in which the novel compositions of the present invention may be incorporated for administration orally or by injection include aqueous solutions, suitably flavored syrups, aqueous or oil suspensions, and flavored emulsions with edible oils such as corn oil, cottonseed oil, sesame oil, coconut oil, or peanut oil, as well as elixirs and similar pharmaceutical vehicles.

Compositions for inhalation or insufflation include solutions and suspensions in pharmaceutically acceptable, aqueous or organic solvents, or mixtures thereof, and powders. The liquid or solid compositions may contain suitable pharmaceutically acceptable excipients as described *supra*. Preferably the compositions are administered by the oral or nasal respiratory route for local or systemic effect. Compositions in preferably pharmaceutically acceptable solvents may be nebulized by use of inert gases. Nebulized solutions may be inhaled directly from the nebulizing device or the nebulizing device may be attached to a face mask tent, or intermittent positive pressure breathing machine. Solution, suspension, or powder compositions may be administered, preferably orally or nasally, from devices which deliver the formulation in an appropriate manner.

The following formulation examples illustrate representative pharmaceutical compositions of the present invention.

Formulation Example 1

Hard gelatin capsules containing the following ingredients are prepared:

Ingredient	Quantity (mg/capsule)
Active Ingredient	30.0
Starch	305.0
Magnesium stearate	5.0

The above ingredients are mixed and filled into hard gelatin capsules in 340 mg quantities.

Formulation Example 2

A tablet formula is prepared using the ingredients below:

Ingredient	Quantity (mg/tablet)
Active Ingredient	25.0
Cellulose, microcrystalline	200.0
Colloidal silicon dioxide	10.0
Stearic acid	5.0

The components are blended and compressed to form tablets, each weighing 240 mg.

Formulation Example 3

A dry powder inhaler formulation is prepared containing the following components:

Ingredient	Weight %
Active Ingredient	5
Lactose	95

The active ingredient is mixed with the lactose and the mixture is added to a dry powder inhaling appliance.

Formulation Example 4

Tablets, each containing 30 mg of active ingredient, are prepared as follows:

Ingredient	Quantity (mg/tablet)
Active Ingredient	30.0 mg
Starch	45.0 mg
Microcrystalline cellulose	35.0 mg
Polyvinylpyrrolidone (as 10% solution in sterile water)	4.0 mg
Sodium carboxymethyl starch	4.5 mg
Magnesium stearate	0.5 mg
Talc	1.0 mg

Total   120 mg

The active ingredient, starch and cellulose are passed through a No. 20
mesh U.S. sieve and mixed thoroughly. The solution of polyvinylpyrrolidone is
mixed with the resultant powders, which are then passed through a 16 mesh U.S.
sieve. The granules so produced are dried at 50° to 60°C and passed through a 16
mesh U.S. sieve. The sodium carboxymethyl starch, magnesium stearate, and talc,
previously passed through a No. 30 mesh U.S. sieve, are then added to the
granules which, after mixing, are compressed on a tablet machine to yield tablets
each weighing 120 mg.

Formulation Example 5

Capsules, each containing 40 mg of medicament are made as follows:

Ingredient	Quantity (mg/capsule)
Active Ingredient	40.0 mg
Starch	109.0 mg
Magnesium stearate	1.0 mg
Total	150.0 mg

The active ingredient, starch, and magnesium stearate are blended, passed
through a No. 20 mesh U.S. sieve, and filled into hard gelatin capsules in 150 mg
quantities.

Formulation Example 6

Suppositories, each containing 25 mg of active ingredient are made as
follows:

Ingredient	Amount
Active Ingredient	25 mg

Saturated fatty acid glycerides to   2,000 mg

The active ingredient is passed through a No. 60 mesh U.S. sieve and suspended in the saturated fatty acid glycerides previously melted using the minimum heat necessary. The mixture is then poured into a suppository mold of nominal 2.0 g capacity and allowed to cool.

Formulation Example 7

Suspensions, each containing 50 mg of medicament per 5.0 mL dose are made as follows:

Ingredient	Amount
Active Ingredient	50.0 mg
Xanthan gum	4.0 mg
Sodium carboxymethyl cellulose (11%)	
Microcrystalline cellulose (89%)	50.0 mg
Sucrose	1.75 g
Sodium benzoate	10.0 mg
Flavor and Color	q.v.
Purified water to	5.0 mL

The active ingredient, sucrose and xanthan gum are blended, passed through a No. 10 mesh U.S. sieve, and then mixed with a previously made solution of the microcrystalline cellulose and sodium carboxymethyl cellulose in water. The sodium benzoate, flavor, and color are diluted with some of the water and added with stirring. Sufficient water is then added to produce the required volume.

Formulation Example 8

Ingredient	Quantity (mg/capsule)
Active Ingredient	15.0 mg
Starch	407.0 mg
Magnesium stearate	3.0 mg
Total	425.0 mg

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The active ingredient, starch, and magnesium stearate are blended, passed through a No. 20 mesh U.S. sieve, and filled into hard gelatin capsules in 425.0 mg quantities.

5

Formulation Example 9

A formulation may be prepared as follows:

Ingredient	Quantity
Active Ingredient	5.0 mg
Corn Oil	1.0 mL

10

Formulation Example 10

A topical formulation may be prepared as follows:

15

Ingredient	Quantity
Active Ingredient	1-10 g
Emulsifying Wax	30 g
Liquid Paraffin	20 g
White Soft Paraffin	to 100 g

20

The white soft paraffin is heated until molten. The liquid paraffin and emulsifying wax are incorporated and stirred until dissolved. The active ingredient is added and stirring is continued until dispersed. The mixture is then cooled until solid.

25

Another preferred formulation employed in the methods of the present invention employs transdermal delivery devices ("patches"). Such transdermal patches may be used to provide continuous or discontinuous infusion of the compounds of the present invention in controlled amounts. The construction and use of transdermal patches for the delivery of pharmaceutical agents is well known in the art. See, e.g., U.S. Patent 5,023,252, issued June 11, 1991, herein

30

incorporated by reference. Such patches may be constructed for continuous, pulsatile, or on demand delivery of pharmaceutical agents.

Frequently, it will be desirable or necessary to introduce the pharmaceutical composition to the brain, either directly or indirectly. Direct techniques usually involve placement of a drug delivery catheter into the host's ventricular system to bypass the blood-brain barrier. One such implantable delivery system used for the transport of biological factors to specific anatomical regions of the body is described in U.S. Patent 5,011,472 which is herein incorporated by reference.

Indirect techniques, which are generally preferred, usually involve formulating the compositions to provide for drug latentiation by the conversion of hydrophilic drugs into lipid-soluble drugs. Latentiation is generally achieved through blocking of the hydroxy, carbonyl, sulfate, and primary amine groups present on the drug to render the drug more lipid soluble and amenable to transportation across the blood-brain barrier. Alternatively, the delivery of hydrophilic drugs may be enhanced by intra-arterial infusion of hypertonic solutions which can transiently open the blood-brain barrier.

Other suitable formulations for use in the present invention can be found in *Remington's Pharmaceutical Sciences*[2].

Utility

The compounds of this invention modulate cellular receptor activity and accordingly, may be used for the treatment of biological conditions in animals. More particularly the compounds may be used in the treatment of medical and veterinary conditions in mammals. They are also useful as insecticides, and for other agricultural application such as crop protection. They are useful as anti-microbials and fungicides.

The compounds of the invention are particularly useful in treating pathological conditions mediated in one form or another by cellular receptor activity. Accordingly, the invention also relates to pharmaceutical composition

comprising a pharmaceutically acceptable excipient and an effective amount of a compound of the invention.

Additionally, the compounds of the invention may be bound to affinity resins for affinity chromatography. The compounds of the invention may be used as a tool in immunoprecipitation. The compounds may be used to identify a receptor *in vitro* for example in microscopy, electrophoresis and chromatography.

In order to further illustrate the present invention and advantages thereof, the following specific examples are given but are not meant to limit the scope of the claims in any way.

EXAMPLES

In the examples below, all temperatures are in degrees Celcius (unless otherwise indicated) and all percentages are weight percentages (also unless otherwise indicated).

Examples 1 - 5 are given as representative examples of methods for preparing the linkers.

In the examples below, the following abbreviations have the following meanings. If an abbreviation is not defined, it has its generally accepted meaning.

Å	=	Angstroms
cm	=	centimeter
DIC	=	2-dimethylaminoisopropyl chloride hydrochloride
DCC	=	*N,N*-dicyclohexylcarbodiimide
DCM	=	dichloromethane
DIPEA	=	diisopropylethylamine
DMA	=	*N,N*-dimethylacetamide
DMAP	=	*4-N,N*-dimethylaminopyridine
DMF	=	*N,N*-dimethylformamide
DMSO	=	dimethylsulfoxide
DPPA	=	diphenylphosphoryl azide

g	=	gram
HBTU	=	1-hydroxybenzotrizole
HPLC	=	high performance liquid chromatography
Hunig's base	=	diisopropylethylamine
MFC	=	minimum fungicidal concentration
mg	=	milligram
MIC	=	minimum inhibitory concentration
min	=	minute
mL	=	milliliter
mm	=	millimeter
mmol	=	millimol
N	=	normal
PyBOP	=	pyridine benzotriazol-1-yloxy-tris(dimethyl-amino)phosphonium hexafluorophosphate
t-BOC	=	tert-butyloxycarbonyl
TBAF	=	tetrabutyl ammonium fluoride
TFA	=	trifluoroacetic acid
THF	=	tetrahydrofuran
μL	=	microliters

EXAMPLE 1

Preparation of [C-C] Compounds of Formula I

(1) Preparation of a Compound of Formula (3) in which m is 2 and n is 3

To a solution of tert-butyl N-(2- aminoethyl)carbamate (2.3g, 14.4 mmol) and N,N-diisopropylethylamine (2.5 mL, 14.3 mmol) in 15 mL methylene chloride at 0°C was added dropwise glutaryl dichloride (0.6 mL, 4.7 mmol) in 15 mL methylene chloride. The resulting mixture was allowed to warm to room temperature with stirring while adding water (15 mL). The methylene chloride was removed under reduced pressure and more water was added (30 mL). The resulting suspension was filtered and washed sequentially with 10% potassium hydrogen sulfate, water, saturated sodium bicarbonate, and water. The solid was dried under vacuum yielding 1.3 g (3.1 mmol, 66%) of pentanedioic acid bis-[(2-t-butoxycarbonylaminoethyl)amide], a compound of formula (3).

Similarly, varying the composition of m and n, other compounds of formula (3) can be prepared.

(2)   Preparation of a Compound of Formula (4) in which m is 2 and n is 3

5   Pentanedioic acid bis-[(2-t-butoxycarbonylaminoethyl)amide], a compound of formula (3) (1.3 g, 3.1 mmol) was suspended in 15 mL methylene chloride. 15 mL of trifluoroacetic acid was added at room temperature giving (with effervescence) a solution that was stirred for 40 minutes, then evaporated *in vacuo*. The residue was dissolved in methanol and treated with 3 mL of 4 N hydrogen chloride in

10   dioxane followed by diethyl ether, giving a gum. The liquids were decanted and the gum dried under vacuum yielding 1.0 g (3.4 mmol,) of pentanedioic acid bis-[(2-aminoethyl)amide], a compound of formula (4).

Similarly, varying m and n, other compounds of formula (4) can be prepared.

15   ## (3)   Preparation of a Compound of Formula I

At room temperature, a carboxyl containing ligand (e.g., amphotericin) (2.3 mmol) is dissolved in 36 mL of DMSO. To this solution is added pentanedioic acid bis-[(2-aminoethyl)amide], a compound of formula (4) (1.0 g, 3.4 mmol) suspended in 27 mL DMF followed by addition of *N,N*-diisopropylethylamine (2.4

20   mL, 13.8 mmol). The resulting suspension is stirred at room temperature for several hours until it is mostly soluble. Then a solution of PyBOP (1.3 g, 2.5 mmol) and 1-hydroxybenzotriazole (310 mg, 2.3 mmol) in 9 mL DMF is added rapidly dropwise. The mixture is stirred at room temperature for 1 hour and then added dropwise to 600 mL of acetonitrile, giving a precipitate that is filtered,

25   washed with acetonitrile ,then diethyl ether, and dried under vacuum. The crude product is purified by reverse phase HPLC (50 minute 2-30% acetonitrile in water containing 0.1% trifluoroacetic acid to yield [C]-pentanedioic acid (2-aminoethyl)amide) ligand and [C-C]-pentanedioic acid bis-[(2-aminoethyl)amide]-bis-(ligand), a compound of Formula I as their respective trifluoroacetic acid salts.

(4)   Preparation of other Compounds of Formula I

Similarly, following the procedures of Example 1, steps 1-3, other compounds of Formula I can be prepared.

5   EXAMPLE 2

Alternative Preparation of [C-C] Compounds of Formula I

(1)   Preparation of a Compound of Formula (7) in which m is 2

At room temperature a carboxyl containing ligand for a cellular receptor containing a carboxyl group (4.7 mmol) is dissolved in 75 mL of DMSO. To this

10   solution is added N,N-diisopropylethylamine (4.1 mL, 23.5 mmol) followed by 9-fluorenylmethyl N-(2-aminoethyl)carbamate hydrochloride (1.8 g, 5.6 mmol). To the resulting solution at room temperature is added rapidly dropwise a solution of PyBOP (2.7 g, 5.2 mmol) and 1-hydroxybenzotriazole (630mg, 4.7mmol) in 75mL 1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone. The resulting solution is

15   stirred at room temperature for 2 hours, then poured into 800 mL diethyl ether, giving a gum. The diethyl ether is decanted and the gum is washed with additional diethyl ether to give a compound of formula (7).

(2)   Preparation of a Compound of Formula (8) in which m is 2

20   The gum of formula (7) is then taken up in 40 mL of DMF, to which 10mL of piperidine is added and the solution left to stand at room temperature for 20 minutes. The solution is then added dropwise to 450 mL of acetonitrile giving a precipitate. Centrifugation is followed by decantation of the acetonitrile and the residue washed twice with 450 mL of acetonitrile, once with 450 mL of diethyl

25   ether and air dried. The residue is taken up in water, acidified to pH < 5 with a small amount of 3 N hydrochloric acid and purified by reverse-phase HPLC using a gradient of 2-30% acetonitrile in water containing 0.1% trifluoroacetic acid yielding a compound of formula (8).

(3)   Preparation of a Compound of Formula I

Compound (8) (400 mg, 220 μmol) and glutaric acid (10 mg, 76 μmol) are dissolved in 5 mL DMF and N,N-diisopropylethylamine (140 μL, 800 μmol) followed by addition of PyBOP (83mg, 160 μmol) and 1-hydroxybenzotriazole (10mg, 74 μmol) in 500 mL DMF. The reaction is stirred for 75 minutes at room temperature then an additional 20 mg of PyBOP is added. 75 minutes later the solution is dripped into 45 mL acetonitrile. The resulting precipitate is collected by centrifugation, washed with ether, air dried and purified by reverse-phase HPLC (50 min 2-30% acetonitrile in water containing 0.1%trifluoroacetic acid, elutes at 33 min) to give a compound of Formula I as its trifluoroacetic salt.

(4)   Preparation of other Compounds of Formula I

Accordingly, following the procedures of Example 2, steps 1-3, other [C-C] compounds of Formula I can be prepared.

EXAMPLE 3

Preparation of a [C-V] Compound of Formula I in which Position C is Substituted

(1)   Preparation of a Compound of Formula (22) in which m and n are both 2

To a solution of an amino containing ligand (26.8 μmol) in DMF (2.0 mL) is added a compound of the formula:

Ligand-C(O)NHCH$_2$CH$_2$NHC(O)CH$_2$CH$_2$C(O)$_2$FM (FM refers to 9-Fluorenyl) (26.8 μmol), followed by PyBOP (20.9 mg, 40.2 μmol), HOBt (5.40 mg, 40.2 μmol), and Hunig's base (23.3 μL, 134 μmol). The reaction solution is stirred for 1 hour and then added dropwise to 20mL of acetonitrile giving a precipitate, which is collected by centrifugation. The crude precipitate is dried in air, yielding a compound of formula (22). The compound is used in the next step without further purification.

(2)   Preparation of a Compound of Formula (23) in which m and n are both 2

The compound of formula (22) is dissolved in 1 mL of DMF, and 100 μL of piperidine is added to the solution. The solution is allowed to stand at room temperature for 30 minutes, following the course of the reaction by mass

5   spectroscopy. The reaction solution is then added dropwise to 20 mL of acetonitrile, giving a precipitate that is collected by centrifugation. The precipitate is purified by reverse phase HPLC on a Ranin C18 Dynamax column (2.5 cm x 25 cm, 8 μm particle size), at 10 mL/min flow rate using 0.045% TFA in water as buffer A and 0.045% TFA in acetonitrile as buffer B (HPLC gradient of 2-50%

10   Buffer B over 90 minutes). The desired product is identified by mass spectroscopy using an API 300 electrospray mass spectrometer and afterwards lyophilized to a white powder to afford compound (23) as a white powder.

Preparation of a Compound of Formula I

15   The compound of formula (23) prepared above (4.80 μmol) is dissolved in 500 μL of DMF. A ligand for a cellular receptor having a free amino group, such as a ligand of formula (17) (4.80 μmol) is added to the solution, followed by PyBOP (2.50 mg, 4.8 μmol), HOBt (0.65 mg, 4.80 μmol) and Hunig's base (6.70 μL, 38.4 μmol). The reaction solution is added dropwise to 20mL of acetonitrile,

20   giving a precipitate that is collected by centrifugation. The precipitate is purified by reverse phase HPLC on a Ranin C18 Dynamax column (2.5 cm x 25 cm, 8 μm particle size), at 10 mL/min flow rate using 0.045% TFA in water as buffer A and 0.045% TFA in acetonitrile as buffer B (HPLC gradient of 2-50% B over 90 minutes). The desired product is identified by mass spectroscopy using an API 300

25   electrospray mass spectrometer.

. EXAMPLE 4

Preparation of a [C-N] Compound of Formula I

(1)   Preparation of a Compound of Formula (24) in which m is 2

A ligand having both primary and secondary amines such as those found in Figure 8 (2.60mmol) is suspended in 40 mL of 1,3-dimethyl-3,4,5,6-tetrahydro-2-(1H)-pyrimidinone and heated to 70°C for 15 minutes. N-(9-fluorenylmethoxycarbonyl)-aminoacetaldehyde (720 mg, 2.6 mmol) is added and

5   the mixture is heated at 70°C for one hour. Sodium cyanoborohydride (160 mg, 2.5 mmol) in 2 mL methanol is added and the mixture is heated at 70°C for 2 hours, then cooled to room temperature. The reaction solution is added dropwise to 20mL of acetonitrile, giving a precipitate that is collected by centrifugation. The precipitate is purified by reverse phase HPLC on a Ranin C18 Dynamax column

10   (2.5 cm x 25 cm, 8 μm particle size), at 10 mL/min flow rate using 0.045% TFA in water as buffer A and 0.045% TFA in acetonitrile as buffer B (HPLC gradient of 10-70% B over 90 minutes), which yielded a compound of formula (24) as its trifluoroacetate salt.

15

(2)   Preparation of a Compound of Formula (25) in which m is 2 and p is 3

The compound of formula (24) obtained above (291 mg, 150 μmol) is dissolved in 3mL of DMF. 3-(dimethylamino)propylamine (28.3 μL, 225 μmol) is added, followed by the addition of PyBOP (85.8 mg, 165 μmol), HOBt (20.3 mg,

20   150 μmol) and Hunig's base (65.0 μL, 375 μmol). The reaction solution is stirred for one hour and then added dropwise to 20mL of acetonitrile giving a precipitate, which is collected by centrifugation. Recovery of this precipitate provides for a compound of formula (25).

25   (3)   Preparation of a Compound of Formula (26) in which m is 2 and p is 3

The compound of formula (25) obtained above is dissolved in 1mL of DMF, and 100 μL of piperidine is added to the solution. The solution is allowed to stand at room temperature for 30 minutes and the course of the reaction is followed by mass spectroscopy. The reaction solution is added dropwise to 20mL of

acetonitrile, giving a precipitate that is collected by centrifugation. The precipitate is purified by reverse phase HPLC on a Ranin C18 Dynamax column (2.5 cm x 25 cm, 8 μm particle size), at 10 mL/min flow rate using 0.045% TFA in water as buffer A and 0.045% TFA in acetonitrile as buffer B (HPLC gradient of 2-50% B

5   over 90 minutes), which yielded the compound of formula (26) as its trifluroacetate salt.

Preparation of a Compound of Formula I

The compound of formula (26) prepared above (3.14 μmol) is dissolved in

10   500 μL of DMF. A compound of formula (19) (3.14 μmol) is added to the solution, followed by PyBOP (2.44 mg, 4.8 μmol), HOBt (0.65 mg, 4.8 μmol) and Hunig's base (6.7 μL, 38.4 μmol). The reaction solution is added dropwise to 20 mL of acetonitrile, giving a precipitate that is collected by centrifugation. The precipitate is purified by reverse phase HPLC on a Ranin C18 Dynamax column

15   (2.5 cm x 25 cm, 8 μm particle size), at 10 mL/min flow rate using 0.045% TFA in water as buffer A and 0.045% TFA in acetonitrile as buffer B (HPLC gradient of 2-50% B over 90 minutes), which yielded a compound of Formula I as its trifluroacetate salt.

20

EXAMPLE 5

Preparation of an [N-N] Compound of Formula I

(1)   Preparation of a Compound of Formula I

The compound of formula (26) prepared above (12.7 μmol) is dissolved in

25   500 μL of DMF. and a compound of the formula:

$HO_2CCH_2CH_2NHOCCH_2CH_2NHOCCH_2CH_2CH_2CONHCH_2CH_2CONHCH_2CH_2CO_2H$ (6.34 μmol) is added, followed by PyBOP (8.24 mg, 15.8 μmol), HOBt (2.13 mg, 15.8 μmol) and Hunig's base (8.8 μl, 51.0 μmol). The reaction solution is added dropwise to 20 mL of acetonitrile, giving a precipitate that is collected by

centrifugation. The precipitate is purified by reverse phase HPLC on a Ranin C18 Dynamax column (2.5 cm x 25 cm, 8 μm particle size), at 10 mL/min flow rate using 0.045% TFA in water as buffer A and 0.045% TFA in acetonitrile as buffer B (HPLC gradient of 2-50% B over 90 minutes), which yielded a compound of formula I as its trifluoroacetate salt.

EXAMPLE 6

Triptan Multivalomer Evaluation

General

Triptan drugs are used in the treatment of migraines. Known pharmaceuticals include Sumatriptan, Zolmitriptan and Naratriptan. These drugs are proposed to have a peripheral and central component to their activity. One explanation is that these agonists of the 5HT1 class of receptors constrict the cerebral vascular system and prevent the plasma extravasation and inflammation that is associated with headache pain. The central effect of these drugs is also believed to operate through inhibition of neurotransmitter release in the trigeminovascular system. The 5HT1d receptors are located in the terminals of the trigeminovascular system. Activity of an agonist at this receptor is believed to inhibit neurotransmitter release and prevent neurogenic inflammation. Another effect is believed to be through vasoconstriction of pathologically dilated cerebrovasculature. It is believed that both receptor agonist activities contribute to the alleviation of the headache pain.

Multimerization

The multimerization positions of triptan include the indole bicyclic core, the C3 substituent and the C5 substituent. (Figures 16-18). The C3 and C5 positions are most likely to tolerate substitution for multimer formation.

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Two similar strategies are followed for the conversion of the pharmacophoric building blocks into symmetrical multivalomers as shown in Figure 18. The "M" in Figure 18 is the site at which the linker is attached to the sumatriptan.

In the first strategy, the building block is reacted with a symmetrical linker

5   molecule to provide the multivalomer. The pharmacophoric building block is an electrophile or a nucleophile. This building block may carry the required reactive functionality at the C3 or the C5 position. The number of molar equivalents of this building block will correlate with the number of complementary reactive sites on the linker molecule.

10   In the second strategy, the building block is reacted first with a spacer group. Then this conjugate is reacted with a symmetrical linker molecule to provide the multivalomer of this invention. The spacer group facilitates the combinatorial generation of multivalomers. (Figure 23)

15   1)   C3 Electrophile (Figures 19 and 21)

The C3 mesylate (700 mg, 2 mmol) is dissolved in $CHCl_3$ (10 ml), DIPEA is then added and the reaction is then heated to 40°C. A solution of the diamine (132 mg, 1 mmol) in DCM (10 ml) is added to the warmed solution over 30 minutes. The reaction is then heated at reflux for two hours and then allowed to cool. The

20   solvent is removed under vacuum. The crude reaction mixture is then treated with aqueous saturated NH_4Cl solution and then extracted with EtOAc (3 x 30 ml). The organic layer is then dried using Na_2SO_4, the drying agent is then filtered off, and the solvent removed in vacuo to provide the crude product. The desired material is then purified from this mixture using reverse phase HPLC.

25

2)   C3 Nucleophile (Figures 19 and 21)

The C3 methylamine (562 mg, 2 mmol) is dissolved in $CHCl_3$ (10 mls), acetic acid (0.5 mls) is then added and the reaction is heated to reflux. Aldehyde (132 mg, 1 mmol) dissolved in DCM (10 mls) is then added dropwise to the

refluxing solution over 60 minutes and the reaction is refluxed for a further 60 minutes. At this point, the NaBH(OAc)$_3$ (222 mg) is added in portions and the reaction is stirred at reflux for a further 2 hours. The reaction is allowed to cool and then is quenched with aqueous NH$_4$Cl solution and the pH of the solution is adjusted to pH 7.0 using either 1 M HCl or NaOH. The product is extracted from this aqueous phase with EtOAc (3 x 30 ml). The organic layer is dried using Na$_2$SO$_4$, the drying agent is then filtered off and the solvent removed *in vacuo* to provide the crude product. The desired material is purified from this mixture using reverse phase HPLC.

3)   C5 electrophile (Figures 20 and 21)

The C5 sulphonate (720 mg, 2.0 mmol) is dissolved in CH$_2$Cl$_2$ (10 ml) and DIPEA is then added. A solution of the 1,3-propandiamine (60 mg, 1 mmol) in CH$_2$Cl$_2$ (10 ml) is then added dropwise over 1 hour via syringe pump. The reaction is then allowed to stir at room temperature for a further hour. The solvent is removed *in vacuo* and the crude reaction mixture is diluted with CHCl$_3$ and then the organic layer is treated with 1 M NaOH. The organic layer is then dried using Na$_2$SO$_4$, the drying agent is then filtered off, and the solvent removed *in vacuo* to provide the crude product. The desired material is then purified from this mixture using reverse phase HPLC.

4)   C5 Nucleophile (Figures 20 and 21)

The C5 sulphonamide (590 mg, 2.0 mmol) is dissolved in DMF (10 ml) and cooled to 0°C in an ice bath. Sodium hydride (48 mg, 2.0 mmol) is then added and the reaction is allowed to stir at this temperature for 1 hour. The dibromide (265 mg, 1 mmol) in a solution in DMF (10 ml) is added dropwise via syringe pump over 1 hour, and the reaction is then allowed to warm to room temperature and stirred for a further 1 hour. The reaction is then quenched with isopropanol (1 ml). The solution is treated with aqueous NH$_4$Cl solution and the pH of the solution

adjusted to pH 7.0 using either 1 M HCl or NaOH. The product is then extracted from this aqueous phase with EtOAC (3 x 30 ml). The organic layer is then dried using Na_2SO_4, the drying agent is then filtered off, and the solvent removed *in vacuo* to provide the crude product. The desired material is then purified from this mixture using reverse phase HPLC.

5)   C3 Nucleophile and C3 Electrophile (Figure 22)

The C3 acid (660 mg, 2 mmol) is dissolved in DMF (10 ml); DIPEA (2 mmol) and DIC (2 mmol) are then added and the reaction is stirred at room temperature for 20 minutes. A solution of the 1,3-propandiamine (74 mg, 1 mmol) in DMF (10 ml) is then added dropwise over 1 hour. The reaction is then allowed to stir at room temperature for a further hour. The reaction is then quenched with saturated aqueous NH_4Cl solution and extracted with ethyl acetate. The organic layer is then dried using Na_2SO_4, the drying agent is then filtered off, and the solvent removed *in vacuo* to provide the crude product. The desired material is then purified from this mixture using reverse phase HPLC.

6)   C5 Nucleophile and C5 Electrophile (Figure 22)

The C5 acid (888 mg, 2 mmol) is dissolved in DMF (10 ml), DIPEA (3 mmol) and DIC (2 mmol) are then added and the reaction is stirred at room temperature for 20 minutes. A solution of the 1,3-propandiamine (74 mg, 1 mmol) in DMF (10 ml) is then added dropwise over 1 hour. The reaction is then allowed to stir at room temperature for over one hour. The reaction is then quenched with saturated aqueous NH_4Cl solution and extracted with ethyl acetate. The organic layer is then dried using Na_2SO_4, the drying agent is then filtered off, and the solvent removed *in vacuo* to provide the crude product. The desired material is then purified from this mixture using reverse phase HPLC.

Screening Assays

The preclinical pharmacology of triptan multivalomers is evaluated using the following assays. The compounds are evaluated to determine the following parameters:

5
    potency at specific receptor subtypes.

    functional activity/potency at these specific receptor subtypes

    functional activity/potency in a reporter gene assay

    functional activity/potency in isolated tissue.

    activity in relevant animal models

10

The following assays are used:

ASSAY GROUP I- Measurement of *in vitro* binding assay/potency using
                radioligand binding assays

ASSAY GROUP II- Evaluation of agonist/partial agonist character in *in vitro*

15
                functional assays.

ASSAY GROUP III-   Measurement of Functional Activity in *in vitro* reporter
                      gene assay.

ASSAY GROUP IV-   Measurement of Efficacy in *ex vivo* functional assays

ASSAY GROUP V- Utilization of *in vivo* models

20

Triptan multi-valency binding agents are evaluated for efficacy by methods similar to the assays that are described in Pramod R.Saxena and Michel Ferrari[3] .

These multi-valency binding agents are also evaluated in assays that are similar to those set forth in G.R. Martin et al.[4], and J. Ngo et al.[5].

25
More specifically triptan multivalomers are evaluated according to the following protocols that have been used to discover and develop currently marketed triptan drugs.

1. ASSAY GROUP I-Measurement of *in vitro* binding assay/potency using radioligand binding assays

Triptan multi-binding agents exhibit potency of 10-0.1 nM at a selection of the following receptors.

The triptan multi-binding agents are evaluated for binding affinity against the human forms of the following serotonin receptor subtypes. The following four receptor subtypes have been selected. This selection does not eliminate evaluation of other serotonin receptor subtypes. Multi-valent binding agents are also evaluated against non-human receptor subtypes to correlate with the *in vivo* screening profile.

A)   5HT1a receptor subtype

A method similar to that set forth in Petrus J. Pauwels et al.[6] and Philippe Schoeffter and Daniel Hoyer[7] is used. These references describe methods by which the effects of a compound is investigated in radioligand binding studies and in functional models for 5-HT1A, 5-HT1B, and 5-HT1D receptors (inhibition of forskolin-stimulated adenylate cyclase activity in calf hippocampus and in rat and calf substantia nigra, respectively) and 5-HT1C receptors (stimulation of inositol phosphate production in pig choroid plexus).

B)   5HT1b receptor subtype

A method similar to that set forth in M.S. Beer et al.[8] is used. The potency of the multi-valent compound is compared with L-694,247 which has an affinity (pIC50) of 10.03 at the 5-HT1D binding site and 9.08 at the 5-HT1B binding site (sumatriptan: pIC50 values 8.22 and 5.94 respectively). L-694,247 retains good selectivity with respect to the 5-HT1A binding site (pIC50 = 8.64), the 5-HT1C binding site (6.42), the 5-HT2 binding site (6.50) and the 5-HT1E binding site (5.66). L-694,247, like sumatriptan, displays a similar efficacy to 5-HT in inhibiting forskolin-stimulated adenylyl cyclase in guinea-pig substantia nigra although L-694,247 (pEC50 = 9.1) is more potent than sumatriptan (6.2) in this 5-HT1D receptor-mediated functional response. L-694,247 (pEC50 = 9.4) is also more potent than sumatriptan (6.5) in a second 5-HT1D receptor mediated

functional response, the inhibition of K+-evoked [3H]-5-HT release from guinea-pig frontal cortex slices.

See also Thierry Wurch et al.[9] for other methods similar to those used to test multi-valent compounds.

C)   5HT1d receptor subtype

A method similar to that set forth in Sarah A. Veldman and Michal J. Bienkowski[10] is used. In this method, expression of the receptor in Chinese hamster ovary cells creates high affinity binding sites for 5HT that is coupled to the inhibition of adenyl cyclase.

D)   5HT1f receptor subtype

A method similar to that set forth in Nika Adham et al.[11] is used to investigate the relation between receptor occupancy and inhibition of cAMP accumulation mediated by 5-HT1F receptors in NIH-3T3 cells (and hence the degree of receptor reserve). This reference shows that a half-maximal response requires only about 10% receptor occupancy, consistent with a receptor reserve of 90% (88%) for 5-HT-induced inhibition of FSCA.

The method for investigating activation of additional signal transduction pathways by the 5-HT1F receptor set forth in this reference is also followed. This reference shows that the responses differ in the two cell lines with respect to stimulation of phospholipase C. The data indicates that the human 5-HT1F receptor can couple to multiple effectors, and that this coupling is cell-type dependent. See also N. Adham et al.[12]

2. ASSAY GROUP II-Evaluation of agonist/partial agonist character in *in vitro* functional assays

A)   5HT1a subtype (functional)

A method similar to that set forth in Petrus J. Pauwels et al.[6] is used to test the forskolin-stimulated c-AMP formation mediated by 5HT1a receptors in CHO-K1 cells.

B) 5HT1b subtype (functional)

A method similar to that described in Petrus J. Pauwels et al.[6] is used.

C) 5HT1d subtype (functional)

A method similar to that described in Petrus J. Pauwels et al.[13] is used to investigate the pharmacology of human serotonin (5-HT)1D receptor sites by measuring two functional cellular responses, inhibition of forskolin-stimulated cAMP formation and promotion of cell growth, using transfected rat C6-glial cell lines and a broad series of 5-HT receptor agonists.

Another method similar to that set forth in John M. Zgombick et al.[14] is used for pharmacological evaluations of serotonergic compounds that inhibit forskolin-stimulated cAMP accumulation in NIH-3T3 fibroblasts (1Dα cell line) and Y-1 adrenocortical tumor cells (1Dβ cell line) stably expressing recombinant human 5-HT1Dα and 5-HT1Dβ receptor subtypes, respectively.

D) 5HT1f receptor subtype (functional)

A method similar to that set forth in Nika Adham et al.[11] is used .

3. ASSAY TYPE III-Measurement of Functional Activity in *in vitro* reporter gene assay.

A method similar to that set forth in S.E. George[15] is used. A cAMP-responsive reporter cell line has been established through the stable expression of a luciferase reporter plasmid in Chinese hamster ovary (CHO) cells. Reporter cells show a dose-dependent expression of luciferase in response to incubation with

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forskolin. These CHO cells are screened for endogenous G protein-coupled receptors capable of stimulating or inhibiting adenylyl cyclase, by monitoring changes in luciferase expression. The response to 5-HT is reversed by the 5-HT1 receptor antagonists cyanopindolol and pindolol, but not the 5-HT2 receptor
5   antagonist ketanserin. This reporter gene assay gives the expected pharmacological profile for these receptors when compared with cAMP accumulation assays, confirming its value as a functional assay for G protein-coupled receptors linked to adenylyl cyclase.

10   **4. ASSAY GROUP IV-Measurement of Efficacy *ex vivo* functional assay**

It is preferable for the compounds to constrict cranial vasculature and not coronary vasculature . Although currently the triptan drugs are believed to provide relief for migraine headache through the constriction of the carotid vasculature, they also demonstrate constrictive effects in the coronary vasculature.

15

Rabbit Saphenous Vein-

A method similar to that set forth in Jean-Pierre Valentin et al.[16] is used to investigate whether contractile responses evoked by multi-valent 5-HT1D receptor agonists are influenced by the endothelium (E) and nitric oxide (NO) in the rabbit
20   isolated saphenous vein. 5-HT, 5-carboxamidotryptamine (5-CT) and sumatriptan (Sum) contract rabbit saphenous vein rings in the potency order (pD2 range) of 5-CT(7.2-7.6) > 5-HT(6.2-7.1) > Sum(5.0-5.8). Efficacy, as assessed by the maximal contractile response (Emax), is significantly greater for Sum compared to 5-HT and 5-CT. In conclusion, the efficacy, but not the potency, of 5-HT, 5-CT
25   and Sum in evoking 5-HT1D receptor-mediated contractile responses is subject to a substantial inhibitory influence of the endothelium and of an EDRF (NO).

Dog Basilar Artery-

A method similar to that set forth in Andre Van de Water et al.[17] is used. In anesthetized dogs, alniditan or (-)-(R)-N-[3,4-dihydro-2H-1-benzopyran-2-ylmethyl]-N'-(1,4,5,6-tetrahydro-2-pyrimidinyl)-1,3-propanediamine dihydrochloride, a compound with 5-HT1-like receptor ligand effects, dose dependently (0.63-80 µg/kg i.v.) reduces common carotid arterial blood flow with comparatively little effect on other cardiovascular variables including coronary, mesenteric and renal arterial blood flow, systemic and pulmonary vascular resistance and airway resistance. The potency of alniditan is higher than that of sumatiptan.

Dog Middle Cerebral Artery-

A method similar to that set forth in F. D. Yocca[18] is used.

Human Middle Cerebral Artery-

A method similar to that set forth in Edith Hamel et al.[19] is used to determine the pharmacological profile of any 5-HT receptor which induces contraction of the bovine isolated cerebral arteries. Several multi-binding 5-HT receptor agonists are tested for their ability to induce vasoconstriction in bovine pial arteries and their potencies will be compared to that of 5-HT. The cerebral vasoconstriction in bovine cerebral arteries is mediated by a receptor homologous to the human cerebrovascular 5-HT1D receptor. Bovine pial arteries appear to be the best available pharmacological model for the human cerebrovascular 5-HT1D receptor.

Human Epicardial Coronary Artery Rings-

A method similar to that set forth in J. Longmore et al.[20] is used. This reference compares the effects of rizatriptan and L-741,519 with those of 5-HT and sumatriptan on endothelium-denuded segments of human coronary artery *in vitro* and confirms that rizatriptan is less effective than sumatriptan in causing contraction of human isolated coronary artery. Furthermore, it shows a the lower

maximum contractile response to rizatriptan, compared with that of sumatriptan.
See also A. Ferro et al.[20] for a method useful in this invention. Both sumatriptan
and MK-462 were significantly less efficacious than 5-HT in contracting human
coronary artery and furthermore MK-462 was significantly less effective than
5   sumatriptan.

5. ASSAY GROUP V-Utilization of *in vivo* models

(a) Inhibition of neurogenic plasma protein extravasation during unilateral electrical
stimulation of the trigeminal ganglion of guinea pigs.

10   A method similar to that set forth in P.R. Saxena[22] is used.

(b) Inhibition of Neuropeptide Release

A method similar to that set forth in P.J. Goadsby and Edvinsson, L.[23] is
used Trigeminal stimulation results in the release of substance P and CGRP.
15   Zolmitriptan reduces neuropeptide release during trigeminal ganglion stimulation in
cats.

EXAMPLE 7

Multivalomers of Muscarinic Antagonists

20

General

Muscarinic receptors are composed of a family of five subtypes (M1-M5)
each of which can be distinguished pharmacologically and structurally. The
physiological role of each subtype in the central and peripheral nervous systems
25   remains to be absolutely clarified.

Several agonists with functional selectivity for M1 receptors may prove
useful in treating Alzheimer's disease. Selective M1/M3 antagonists may prove
useful in the treatment of disorders of smooth muscle function.

Three subtypes of muscarinic receptor are found in human airways:

M1-receptors facilitate ganglionic transmission and therefore enhance cholinergic reflexes.

M2-receptors are localized to post-ganglionic cholinergic nerve terminals and inhibit the release of acetylcholine

5   M3-receptors on airway smooth muscle mediate constriction and on submucosal glands mediate increased mucus secretion.

M3 or mixed M1/M3-receptor antagonists are preferable since they would not increase acetylcholine release from cholinergic nerves.

Muscarinic receptor subtypes: pharmacology and therapeutic potential are

10   described in Richard M. Eglen and Sharath S. Hegde[24]. Selective muscarinic receptor agonists and antagonists are described in Richard M. Eglen and Nikki Watson[25].

U. Holzgrabe et al.[26] describes allosteric small molecule binding sites for the muscarinic acetylcholine receptors.

15   Tropium drugs for airway disease include Ipratropium, Oxitropium, Tiotropium bromide and Revatropate.

a)   Ipratropium bromide (ATROVENT) is a quaternary ammonium compound formed by the introduction of an isopropyl group to the N atom of atropine. Ipratropium bromide is often more effective than β_2-adrenergic agonists in the

20   treatment of patients with chronic obstructive pulmonary disease (where cholinergic tone is usually the only reversible component), but is less effective than β_2-adrenergic agonists in the treatment of asthma.

Parenteral administration causes bronchodilatation, tachycardia, and inhibition of salivary secretion but lacks significant effects on the CNS. A

25   therapeutically important property of ipratropium is the relative lack of effect on mucociliary clearance. The use of ipratropium in airway disease avoids the increased accumulation of lower airway secretions and the interference of β-adrenergic agonist-induced enhancement of mucociliary clearance

After inhalation, maximal responses usually develop over 30 to 90 minutes, and significant effects may persist for more than 4 hours. Ipratropium has minimal systemic effects since its quaternary structure limits absorption through the mucous membrane of the respiratory and gastrointestinal tracts. The bronchodilation produced by ipratropium in asthmatic subjects develops more slowly and is usually less intense than that produced by adrenergic agonists. Some asthmatic patients may experience a useful response lasting up to 6 hours. The bronchodilation produced by ipratropium is primarily a local, site-specific effect rather than a systemic effect. Ipratropium appears to produce bronchodilation by competitive inhibition of cholinergic receptors on bronchial smooth muscle.

b)   Oxitropium bromide is similar to ipratropium.

c)   Tiotropium bromide

The most recently developed and bronchoselective member of this family is tiotropium bromide which has a longer duration of action.

d)   Revatropate

See A. M. Martel[27] for a description. The treatment of airway obstructive disease may be improved by antimuscarinic agents which selectively block M1 and M3 receptors but do not inhibit prejunctional cholinergic autoreceptors which limit release of acetylcholine. Revatropate is a antimuscarinic agent which shows some 50-fold selectivity for M1 and M3 receptors in guinea pig trachea and rabbit vas deferens over the M2 subtype in atria. This selectivity profile was seen *in vivo* in anesthetized guinea pigs and conscious dogs where bronchodilator activity was produced in the absence of any effect on heart rate. Revatropate, in contrast to the non-selective agent ipratropium, does not potentiate bronchoconstrictor responses induced by vagal nerve stimulation, indicating that inhibitory autoreceptors were still functional. Early clinical studies in COPD patients showed that inhaled revatropate was an effective bronchodilator which was well tolerated.

Method for Ipratropium Multivalomerization

Ipratropium is covalently attached to linkers by the following two methods.

i) Ipratropium may be attached via functional groups located within the drug

(pharmacophoric building blocks) e.g. the hydroxyl group of the aldol product, the

5   C-N bond of the tropane ring system.   See Figures 25 and 26 which set forth the

preferred sites for attachment.

ii) Alternatively Ipratropium may be attached to a linker via a functional group

previously introduced into the aromatic ring to facilitate multivalomer construction.

Suitable functional groups include -Br, -NH$_2$, -OH, -CO$_2$H, and -CHO. These

10   functional groups may also require a suitable spacer between this group and the

main element of the pharmacophore.

Figure 27 illustrates specific linkers for use with a functionality already

present within the ipratropium.

Figure 28 illustrates the different valencies of the multivalomers that may be

15   used. Dimers, trimers and tetramers are exemplified. These are all homovalomers

using the same point of attachment within the ligand.

Figure 29 illustrates the role the linker/framework core plays in governing

the spatial, physicochemical, pharmacological and pharmacokinetic profiles of

these multivalomers. "n" defines the valency of the multivalomer. "0" defines the

20   linker/framework core and → identifies the differing points of attachment and

orientation of the pharmacophore.

Multimers are constructed by the following methods.

1.   Quaternization to provide N-linked dimeric Ipratropium (Figure 30)

25   a)   Preparation of the ester(3)

With reference to Figure 30, the acid (1) (256 mg, 1 mmol) is dissolved in

DMF (4 ml) and treated with DIC (126 mg, 1 mmol) and catalytic DMAP (5 mg).

The reaction is stirred at room temperature for 30 minutes. A solution of the

alcohol (2) (169 mgs, 1 mmol) in DMF (4 mls) is added dropwise and the reaction

is stirred at room temperature for 12 hours. After this time the reaction is concentrated *in vacuo* and worked up using standard methods. One of these methods is to partition the crude reaction mixture between ethyl acetate (25 mls) and water (25 mls). The aqueous layer is extracted with ethyl acetate (25 mls x 2). The combined organic layers are dried ($MgSO_4$), filtered and concentrated in vacuo. This mixture is further purified using flash chromatography or HPLC to provide the desired ester(3).

b)   Preparation of the dimer(5)

The tertiary amine(3) (404 mg, 1 mmol) is dissolved in the $CHCl_3$ (10 ml) and is heated to reflux. A solution of the dibromide(4) (180 mg, 0.5 mmol.) is added dropwise in $CHCl_3$ (10 ml) over three hours and the reaction is heated at reflux for another 2 hours. The reaction is allowed to cool and concentrated *in vacuo*. The crude reaction mixture is partitioned between ethyl acetate (25 mls) and water (25 mls). The aqueous layer extracted twice with ethyl acetate (25 ml). The combined organic layers are dried ($MgSO_4$), filtered and concentrated *in vacuo*. This mixture is further purified using flash chromatography or HPLC to provide the desired dimer(5).

c)   Hydrogenolytic removal of protecting groups to provide (6)

Pd/C (10%)(25 mg) is suspended in ethyl acetate (15 ml) under an inert atmosphere of nitrogen. The suspension is thoroughly degassed *in vacuo* and re-equilibrated with nitrogen. This is carried out three times. The reactor is treated with H_2 gas and the catalyst is allowed to take up H_2 for 20 minutes. The protected dimer (5) (1018 mg, 1 mmol) is dissolved in ethyl acetate (5 ml) and this solution is introduced into the catalytic hydrogenation reactor. The reaction is allowed to stir under hydrogen for 12 hours. After this time, the remaining H_2 gas is removed *in vacuo*, and the catalyst is filtered off under N_2 through a pad of Celtite. The ethyl acetate solution is concentrated *in vacuo* and the reaction is purified by HPLC to provide the desired dimer material (6).

2. N-linked Ipratropium dimers from reductive amination (Figure 31)

a) Synthesis of Ester(3)

With reference to Figure 31, a stirred solution of the acid (1) (280 mg, 1 mmol) in DCM (10 ml) is treated with DIC (126 mg, 1 mmol) and catalytic DMAP (5 mol%). The reaction is stirred at room temperature for 30 minutes, treated with a solution of the alcohol(2) (262 mg, 1 mmol) and the reaction is stirred at room temperature for twelve hours. The reaction is allowed to cool and concentrated *in vacuo*. The crude reaction mixture is partitioned between ethyl acetate (25 ml) and water (25 ml). The aqueous layer is extracted twice with ethyl acetate (25 ml). The combined organic layers are dried ($MgSO_4$), filtered and concentrated *in vacuo*. This mixture is further purified using flash chromatography or HPLC to provide the desired ester(3).

b)   Deprotection of the CBZ protecting group to provide amine (4)

Pd/C (10%)(25 mg) is suspended in ethyl acetate (15 ml) under an inert atmosphere of nitrogen. The suspension is thoroughly degassed *in vacuo* and re-equilibrated with nitrogen. This is carried out three times. The reactor is treated with H_2 gas and the catalyst is allowed to take up H_2 for 20 minutes. The protected ester (3) (520 mg, 1 mmol) is dissolved in ethyl acetate (5 ml) and this solution is introduced into the catalytic hydrogenation reactor. The reaction is allowed to stir under hydrogen for 12 hours. After this time the remaining H_2 gas is removed *in vacuo*, and the catalyst is filtered off under N_2 through a pad of Celtite. The ethyl acetate solution is concentrated *in vacuo* and the reaction is purified by HPLC to provide the desired amine(4).

c)   Reductive Amination to provide dimer (6)

The amine (4) (389 mg, 1.0 mmol) is dissolved in $CHCl_3$ (10 ml) and is treated with AcOH (50 μl) and $NaBH(OAc)_3$ (844 mg, 4 mmol) and the reaction is stirred at room temperature for 12 hours. The dialdehyde(5) (70 mg, 0.5 mmol) is added and the reaction is stirred at room temperature for 12 hours. The reaction is quenched with methanol (10 ml) and concentrated *in vacuo*. The crude reaction

mixture is partitioned between ethyl acetate (25 ml) and water (25 ml). The aqueous layer extracted twice with ethyl acetate (25 ml). The combined organic layers are dried (MgSO$_4$), filtered and concentrated *in vacuo*. This mixture is further purified using flash chromatography or HPLC to provide the desired dimer

5   (6)

d)   Methylation of dimer (6) to provide qauternary compound (7).

A stirred solution of the dimer (6) (880 mg, 1 mmol) in CHCl$_3$ (10 ml) is treated with methyl bromide (2 equivalents, 376 mg, 4 mmol) and the reaction is heated at reflux for 12 hours. The reaction is concentrated *in vacuo* and the crude

10   reaction mixture is partitioned between ethyl acetate (25 ml) and water (25 ml). The aqueous layer extracted twice with ethyl acetate (25 ml). The combined organic layers are dried (MgSO$_4$), filtered and concentrated *in vacuo*. This mixture is further purified using flash chromatography or HPLC to provide the desired quaternised dimer (7).

15   e)   Deprotection of the dimer (6) to provide the ipratropium dimer (8)

A solution of the dimeric silyl ether (7) (910 mg, 1 mmol) in anhydrous THF (10 ml) is treated with 1M TBAF in THF (2 ml) and the reaction is allowed to stir at room temperature for 1 hour. After this time the reaction is concentrated *in vacuo*. The crude reaction mixture is partitioned between ethyl acetate (25 ml) and

20   water (25 ml). The aqueous layer is extracted twice with ethyl acetate (25 ml). The combined organic layers are dried (MgSO$_4$), filtered and concentrated *in vacuo*. This mixture is further purified using flash chromatography or HPLC to provide the desired quaternised dimer (8).

25   3)   O-Linked Ipratropium dimers from etherification (Figure 32)

a)   Conversion of the amine (1) to the dimer (3).

With reference to Figure 32, a stirred solution of the alcohol (1) (614 mg, 2 mmol) is dissolved in DMF (10 ml) and is treated with Cs$_2$CO$_3$ (650 mg, 2 mmol) and the reaction is stirred at room temperature for 30 minutes. After this time, a

solution of the bromide (2) (257 mg, 1 mmol) in DMF (5 ml) is added dropwise to the solution over two hours. The reaction is stirred at room temperature for a further two hours. The reaction is concentrated *in vacuo* and the crude reaction mixture is partitioned between ethyl acetate (25 ml) and water (25 ml). The

5   aqueous layer is extracted twice with ethyl acetate (25 ml). The combined organic layers are combined, dried ($MgSO_4$), filtered and concentrated *in vacuo*. This mixture is further purified using flash chromatography or HPLC to provide the desired dimer(3).

b) Conversion to the dimeric quaternary salt (3)

10   The dimer (3) (732 mg, 1 mmol) is dissolved in $CHCl_3$ (10 ml) and is treated with MeBr (188 mg, 2 mmol) and the reaction is refluxed for 12 hours. The reaction is allowed to cool. The reaction is concentrated *in vacuo* and the crude reaction mixture is partitioned between ethyl acetate (25 ml) and water (25 ml). The aqueous layer is extracted twice with ethyl acetate (25 ml). The combined

15   organic layers are dried ($MgSO_4$), filtered and concentrated *in vacuo*. This mixture is further

purified using flash chromatography or HPLC to provide the desired dimeric quaternary species (3).

20   <u>4) O-linked Ipratropium dimers from conjugate addition. (Figure 33)</u>

a) Formation of the ether (3)

With reference to Figure 33, a solution of the acrylate (1) (299 mg, 1 mmol) in DMF (10 ml) is treated with a solution of the cesium alkoxide of the alcohol (2). This alkoxide is prepared by the treatment of the alcohol (2) (196 mg, 0.5 mmol)

25   with Cs_2CO_3 (325 mg, 1 mmol) in DMF (10 ml). The reaction is heated to reflux for twelve hours allowed to cool to room temperature. The reaction is concentrated *in vacuo* and the crude reaction mixture is partitioned between ethyl acetate (25 ml) and water (25 ml). The aqueous layer is extracted twice with ethyl acetate (25 ml). The combined organic layers are dried ($MgSO_4$), filtered and concentrated *in*

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vacuo. This mixture is further purified using flash chromatography or HPLC to provide the desired ether (3).

b)   Hydrogenolysis of the O-Bn protecting group to provide (4).

Pd/C (10%)(25 mg) is suspended in ethyl acetate (15 ml) under an inert atmosphere of nitrogen, the suspension is thoroughly degassed *in vacuo* and re-equilibrated with nitrogen. This is carried out three times. The reactor is treated with H_2 gas and the catalyst is allowed to take up H_2 for 20 minutes. The protected alcohol (3) (500 mg, 1 mmol) is dissolved in ethyl acetate (5 ml) and this solution is introduced into the catalytic hydrogenation reactor. The reaction is allowed to stir under hydrogen for 12 hours. After this time the remaining H_2 gas is removed *in vacuo*, and the catalyst is filtered off under N_2 through a pad of Celtite. The ethyl acetate solution is concentrated *in vacuo* and the reaction is purified by HPLC to provide the desired alcohol (4).

c)   Dimerization though Michael Addition to provide dimer (5)

A solution of the acrylate (1) (299 mg, 1 mmol) in DMF (10 ml) is treated with a solution of the alkoxide (4) (405 mg, 1 mmol) and the reaction is stirred at room temperature for 12 hours. The solution of the alkoxide of (4) is prepared by the treatment of the alcohol (4) in DMF (5 ml) with Cs_2CO_3 (325 mg, 1 mmol) and this solution is stirred at room temperature for 30 minutes. The reaction is concentrated *in vacuo* and the crude reaction mixture is partitioned between ethyl acetate (25 ml) and water (25 ml). The aqueous layer is extracted twice with ethyl acetate (25 ml). The combined organic layers are dried ($MgSO_4$), filtered and concentrated *in vacuo*. This mixture is further purified using flash chromatography or HPLC to provide the desired ether (5).

d)   Quaternization of the dimer to provide (6)

The dimer(5) (704 mg, 1 mmol) is dissolved in $CHCl_3$ (10 ml) and is treated with MeBr (188 mg, 2 mmol) and the reaction is refluxed for 12 hours. The reaction is allowed to cool, The reaction is concentrated *in vacuo* and the crude

reaction mixture is partitioned between ethyl acetate (25 ml) and water (25 ml). The aqueous layer is extracted twice with ethyl acetate (25 ml). The combined organic layers are dried ($MgSO_4$), filtered and concentrated *in vacuo*. This mixture is further purified using flash chromatography or HPLC to provide the desired

5   dimeric quaternary

species (6).

Evaluation of Multimers

These muscarinic multivalomers are then evaluated for their ability to deliver

10   a muscarinic antagonist for the treatment of airway disease. This evaluation is carried out within the framework of the following screening strategy.

i) Potency-M1-M5 *in vitro* radioligand binding assay.

The affinity of the multivalomers is measured on all five muscarinic subtypes

15   of receptor. This provides pKi for the multivalomer ligands at these receptors. This is carried out for the recombinant human receptors and for species that are used in animal models.

Preferably, the multivalomer has a 1-10 nm affinity at the M1 and M3 receptors but an affinity of less than 100 nm at the other three receptors (M2, M4,

20   M5).

These affinities are measured by methods similar to the protocols described by Buckley *et al*[28]. The 5 cholinergic muscarinic receptor genes (m1, m2, m3, m4, and m5) have been identified and cloned. The pharmacological properties of the individual muscarinic receptors have been described. Each of these genes has

25   been transfected into CHO cells (CHO-K1), establishing stable cells lines expressing each receptor. The antagonist binding properties of each muscarinic receptor have been studied.

ii) Efficacy-M1-M5 *in vitro* radioligand binding/functional assay.

The functional activity of the multivalomers are established on the five human muscarinic receptor subtypes. This provides a pKa for the multivalomers at each of these receptors. Preferably, the multivalomers are antagonists and show a pKa of 1-10nM at the M1 and M3 receptors. These multivalomers show significantly reduced affinity (pKa < 100nM) at the M2, M4, and M5 receptors. Required multivalomers do not show any functional agonist or partial agonist activity at these five muscarinic receptors.

These pharmacological properties are measured according to the procedures described by Jyrki P. Kukkonen et al.[29] and Jyrki P. Kukkonen[30].

iii) Potency/Efficacy-M1-M5 *ex vivo* functional studies.

The multivalomers that display selective muscarinic antagonist properties at the M1 and M3 receptor subtypes are evaluated for their functional activity in the *ex vivo* models. This evaluates the ability of the multivalomers to antagonize the acetylcholine induced bronchoconstriction in isolated animal airway tissue. The method is similar to that set forth in J. Garssen et al.[31]. Methods to evaluate multi-binding muscarinic compounds are also set forth in Jennifer Maclagan and Peter J. Barnes[32].

iv) *In vivo* assays.

The *in vivo* methods used to evaluate the M3 selective antagonist are similar to the methods that have been developed to compare the properties of Revatropate with Ipratropium. See for example V.A. Alabaster[33].

Antimuscarinic multivalomers which show M1 and M3 receptor selectivity are evaluated in guinea pig trachea (M1/M3) and rabbit vas deferens (M1/M3) over the atria (M2). This selectivity profile is evaluated in guinea pigs and dogs where bronchodilator activity is produced in the absence of any effect on heart rate.

EXAMPLE 8

AT1 Receptor Screening Strategy Based on Losartan.

The renin-angiotensin system plays a major role in the regulation of the blood pressure and renal function. The 8-mer angiotensin II peptide exerts its effects on
5 blood pressure through mechanisms that include increasing salt and water absorption from the blood in the kidney. Interruption of the renin-angiotensin system through inhibition of the angiotensin converting enzyme (ACE) through the use of small molecule ACE inhibitors has shown clinical benefit in the treatment of the hypertension, congestive heart failure and renal disease. However, the ACE
10 inhibitors generate a dry cough side effect because they also interfere with kinin metabolism.

Antagonism of the cell surface receptor for the angiotensin II peptide is an alternative means of treating hypertension through modulation of the renin-angiotensin system. The major clinical advantage of this approach over ACE
15 inhibitors is that it does not have the dry cough side-effect associated with the ACE inhibitors. See D. J. Dzielak[64], Wexler et al.[65], Clellan and Balfour[66], Burnier and Brunner[67], Merlos et al.[68] and M. Merlos et al.[69].

There are two receptor subtypes for the Angiotensin II octapeptide. These are the AT1 and AT2 receptor subtypes both of which belong to the seven
20 transmembrane superfamily of cell surface receptor. The relevant pathophysiological effects of angiotensin II are exerted through specific agonism of the AT1 receptor.

The AT1 receptor has a high affinity for the sartan drugs and a low affinity for PD 123177. The AT2 receptor has a high affinity for PD 123177 but a low affinity for
25 losartan. It is of interest to note that angiotensin II shows no selectivity for the AT subtypes. The AT1 receptor is a G protein-coupled receptor of 359 amino acids. The AT2 receptor is 363 amino acids. These receptors have little sequence homology (only 32% amino acid sequence identity). The majority of the physiological effects of angiotensin II appear to be mediated by the AT1 receptor,

and no functional role for the AT2 receptor has been defined (Bergsma et al.[70] and Mukoyama et al.[71]).

Losartan and Valsartan are two AT1 antagonists (Figure 34). Other AT1 antagonists include Irbesartan, Candesartan, Eprosartan, Tasosartan, Telmisartan and Ripisartan (Figures 35 and 36).

The AT1 receptor has a small molecule antagonist binding site and a peptide agonist binding site which are partially overlapping. There is an overlap between the propylimidazole and biaryl tetrazole motifs with the side chains of the Ile5-His6-Pro7-Phe8 and the terminal carboxylate in the N-terminus of the angiotensin II peptide.

The residues in the receptor involved in the AT1 receptor-ligand complex may be divided into two groups, those that contribute to the binding of the ligand and those that are involved in signal transduction.

Receptor residues involved in binding peptide agonists.

Lys 199 (tm5) has been identified as the key receptor residue for the binding of agonists and antagonists to the AT1 receptor. This residue has been implicated in the binding of the AII peptide but also appears to be involved in the binding of the phenyl tetrazole or phenyl carboxyl residues of the non-peptide antagonists. Lys 102 (tm3), Arg 167 (EC2), His 183 (EC2) and Asp 263 (EC3) have been proposed as the possible residues for the interaction with the Asp1 and Arg2 of Angiotensin II. These residues show a limited effect on losartan binding.

Receptor residues involved in the binding of non-peptide antagonists.

The non-peptide antagonists bind to a region that extends from the fifth to the seventh transmembrane helix. This is the same general region of the GPCR class of proteins that is occupied by small molecule biogenic amines such as the catecholamines for the β_2-receptor. Residues in transmembrane helices 3-7 that contribute to the binding of the non-peptide antagonists do not play role in the

binding of the endogenous peptide agonist.   It remains unclear whether the binding of these small molecule agonists (e.g. L-162,313) that resemble the small molecule antagonists takes place in the peptide agonist or non-peptide antagonist binding regions. Mutations that are known to affect the binding of losartan and

5   angiotensin II have no effect on the binding of L162,313.

The above information of the specifics of the overlap between the antagonist-agonist binding site is used for the construction of heterovalomers.

Method for multivalomerization of angiotensis II antagonists

10   The six antagonists exemplified contain a biaryl containing an acidic group such as carboxylate, a sulphonamide or isosteric tetrazole (eprosartan is the exception in that it does not contain this motif).   The six antagonists also contain a heterocyclic scaffold at the top of the molecule. This is a region of the molecule that appears to be readily varied and is a site for multivalomer construction (e.g.

15   the imidazole group of losartan). The heterocyclic scaffold is generally substituted with a lipophilic alkyl group, e.g. the propyl group of losartan, and a hydrogen bond acceptor e.g. carboxamide, carboxaldehyde, carboxylate and hydroxymethyl.

At this C2 position of the imidazole, a linear alkyl or alkenyl group is preferred, which is preferably from 3 - 4 carbon atoms. At the C4 position of the

20   imidazole there is a chlorine atom which tolerates large substituents. This is a position that will tolerate connection to the framework building blocks to construct multivalomers. At the C5 position of the imidazole, a variety of substituents are acceptable. A hydrogen bond acceptor, such as carboxamide, carboxaldehyde, carboxylate and hydroxymethyl, is preferred.

25   Consideration of the partially overlapping binding sites has indicated that it is possible to construct heterovalomers derived from losartan and angiotensin II.
i) functional groups are those groups within the drug (pharmacophoric building blocks) that may be exploited for multi-binding compound formation e.g. the tetrazole NH or the primary hydroxyl of losartan.   .

ii) atoms are points were a functional group may be introduced to facilitate multivalomer construction. For example, the introduction of such functional groups into the aryl ring of the biaryl functionality and the replacement of the Cl of the imidazole with a functional group that could be used for the construction of

5   multivalomers. In the areas/regions of the molecule where there is no obvious chemical functional group to use for multi-binding compound construction (ii above) suitable functional groups can readily be introduced e.g -Br, -NH2, -OH, -CO2H, -CHO. These functional groups may also require a suitable spacer between this group and the main element of the pharmacophore.

10   Figures 38 and 39 shows the different points of attachment for specific losartan multivalomers, using functional groups already present within the ligand.

Figure 40 shows possible differing valencies of the multi-binding compounds. These are all homovalomers using the same point of attachment within the ligand.

Figure 41 shows the differing framework cores that could be used.

15   Figure 42 shows the differing orientation of binding elements within the multivalomer.

Figure 43 shows the heterovalomers that are possible within the AT1 antagonist manifold.

Synthesis of 1-hydroxyl linked losartan multivalomer (Figure 44)

20   a.   Alkylation of the imidazole (1) to provide (2)

The procedure for the synthesis of the TBS protected imidazole(1) is set forth in Greenlee[72].

With reference to Figure 44, the imidazole (1) (604 mg, 2mmol) in DMF (10 ml, 0.2M) is treated with NaH (48 mg, 2mmol) and the reaction is stirred at room

25   temperature for 30 minutes. The biaryl bromide (300mg, 1mmol.) is then added as a solution in DMF (5ml) and the reaction stirred for a further 60 minutes. The reaction is concentrated *in vacuo*. The crude reaction mixture is partitioned between ethyl acetate (25 ml) and water (25 ml). The organic layer is dried

(MgSO$_4$), filtered and concentrated *in vacuo*. Flash chromatography provides the desired material (2).

b. Deprotection of the TBS ether (2) to provide the alcohol (3)

The silyl ether (2) (1.02 g, 2mmol) is dissolved in THF (10 mls, 0.2M) and 1M TBAF in THF (3 ml, 3mmol) is added arid the reaction is allowed to stir at room temperature for 2 hours. The reaction is concentrated *in vacuo*, and partitioned between ethyl acetate (25 ml) and water (25 ml). The organic layer is dried (MgSO^4), filtered and concentrated *in vacuo*. The crude reaction mixture is purified by flash chromatography to provide the desired material (3).

c. Dimerization via the primary hydroxyl of (3) to provide the multivalomer (4)

The primary alcohol (3) is dissolved in DMF (10ml, 0.2M) and cooled to 0°C, NaH (48mg, 2mmol) is added and the reaction is stirred at this temperature for 30 minutes. The dibromide (260 mg, 1mmol) is dissolved in DMF (10ml) and is added to the alkoxide solution via syringe pump over 60 minutes. The reaction is concentrated *in vacuo*, and partitioned between ethyl acetate (25 ml) and water (25 ml). The organic layer is dried (MgSO$_4$), filtered and concentrated *in vacuo*. This crude reaction mixture is purified by flash chromatography to provide the desired multivalomer (4).

d. Deprotection of the dimeric tetrazole (4) to the losartan multivalomer (5).

The dimeric protected tetrazole (1.17g,1mmol) is dissolved in methanol (5ml, 0.2M) and is treated with 1M HCl in methanol (3ml, 3mmol) and the reaction is stirred at room temperature for 60 minutes. After this time, the reaction is concentrated *in vacuo*. The reaction is partitioned between ethyl acetate (25 ml) and water (25 ml). The organic layer is separated, dried (MgSO$_4$), filtered and concentrated *in vacuo*. The crude reaction mixture is purified by flash chromatography to provide the losartan multivalomer (5).

Synthesis of 2-hydroxyl linked losartan multi-binding compound (Figure 45)

This is a strategy that is used to prepare either hydroxyl linked or tetrazole linked multivalomers.

a. Alkylation of imidazole (1)

With reference to Figure 45, the imidazole (1), (276 mg, 2mmol) is added to a stirred solution of sodium methoxide in methanol (2mmol, 46mgs of sodium dissolved in (10 ml) methanol) at 0°C. The solvent is removed *in vacuo* and the so formed sodium salt of the imidazole is dissolved in DMF (10 ml). The biaryl bromide (542 mg, 2 mmol) is added and the reaction is stirred at room temperature for 12 hours. The solvent is then removed *in vacuo*, and the reaction partitioned between ethyl acetate (25 ml) and water (25 ml). The organic layer is combined and the organic layer is dried with $MgSO_4$, the solvent in this removed *in vacuo*. Flash chromatography of the crude reaction mixture provides the alkylated imidazole (2).

b. Alkylation of alcohol (2) to provide dimer (3)

Sodium hydride (48 mg, 2mmol) is dissolved in DMF (10ml) and the alcohol (2) (760 mg, 2mmol.) is added with stirring. This reaction is allowed to stir at room temperature. The benzylic dibromide (261 mg, 1mmol) in DMF is added dropwise via syringe pump over two hours. The reaction is allowed to stir at room temperature for a further two hours. The reaction is treated with aqueous NH_4Cl solution and partitioned between ethyl acetate (25 ml) and water (25 ml). The organic layer is separated, dried with $MgSO_4$, filtered and concentrated *in vacuo*. This crude reaction mixture is purified by flash chromatography to provide the pure dimer (3).

c. Conversion of the dimeric nitrile (3) to dimeric tetrazole (4)

The dimer (3) (430 mg, 1 mmol) is dissolved in xylene (20 ml) and the trimethylstannyl azide is added (615 mg, 3 mmol) and the reaction is heated to reflux in xylene (20 ml) for 24 hours. The solvent is removed *in vacuo* and the crude reaction mixture is treated with 2N NaOH in methanol (20 ml) to remove the N-stannyl group. The solvent is removed *in vacuo* and the reaction is dissolved in water and the solution neutralized (pH=7). The product is extracted with ethyl

acetate (25mls x 3). The organic layer is dried with $MgSO_4$, filtered and concentrated *in vacuo*. The crude reaction mixture is purified by flash chromatography to provide the desired dimeric tetrazole (4).

5   Synthesis of tetrazole linked losartan multivalomer (Figure 46)

a.   Conversion of the nitrile (1) to the tetrazole (2)

For the preparation of nitrile (1), see Figure 45. With reference to Figure 46, the biaryl nitrile (1) (380 mg, 2mmol) is dissolved in xylenes (10 ml) and the trimethylstannyl azide (820 mg, 4mmol) is added. The reaction is heated at reflux

10   for 24 hours. The reaction is allowed to cool and the solvent is removed *in vacuo*. The crude reaction mixture is treated with 1N NaOH in methanol (20 ml) to hydrolyse the N-stannyl bond. The methanol is removed *in vacuo*, the crude reaction mixture is dissolved in water and neutralized with 1M HCl. The product is extracted from the aqueous phase with ethyl acetate (3 x 25 ml). The organic layer

15   is dried with $MgSO_4$, the drying agent is filtered, and the solvent is removed *in vacuo*. The crude reaction mixture can be purified by flash chromatography to provide the desired tetrazole (2).

b.   Conversion of the tetrazole (2) to the dimer (3)

The tetrazole (2) (844 mg, 2mmol) is dissolved in DMF (5 ml) and is treated

20   with NaH (48 mg, 2mmol) and the reaction is stirred at room temperature for 20 minutes. The dibromide alkylating agent (260 mg, 1mmol) in DMF (10 ml) is added to tetrazole solution via syringe pump over one hour. The reaction is allowed to stir at room temperature for a further hour. The reaction is concentrated *in vacuo*, and is partitioned between ethyl acetate (25 ml) and water (25 ml). The

25   organic layer is separated, dried ($MgSO_4$), filtered and concentrated *in vacuo*. The crude reaction mixture is purified by flash chromatography to provide the desired dimer (3).

See also Rivero et al.[73], Duncia et al.[74], Carini et al.[75], and Carini et al.[76]

<u>Assays</u>

The following assays are used to evaluate multi-valomers derived from Angiotensin II (AT1 subtype) receptor antagonists. Where there is a specific protocol that has developed for testing a specific AT1 receptor agonist, this protocol is followed. However, other assay protocols described for a different AT1 receptor antagonist may also be used, eg testing a losartan multimer using a protocol developed for valsaratan.

The method used to evaluate efficacy for hypertension is similar to that set forth in D. J. Dzielak[46] which describes the pre-clinical and clinical pharmacology associated with the seven AT1 receptor antagonists.

The duration of action of the multivalomeric compounds is determined from the PK profile.

Assays similar to the following assays that were utilized in the discovery and development of Losartan, are used in the discovery and development of AT1 targeted multivalomers.

In vitro binding assay.

This assay is used to determine the pKi for the AT1 multi-binding compounds. Measurement of IC_{50} in rat adrenal cortical microsomes in a competition radioligand binding assay with radiolabelled angiotensin II is used. Losartan inhibits the specific binding of ^{125}I-angiotensin II to its receptor sites in rat adrenal cortical membranes and in cultured rat smooth muscle cells.

The method is similar to those set forth in Bergsma et al.[70], and Mukoyama et al.[71].

In vitro functional activity.

Functional activity is measured by the ability of the multi-binding compound to inhibit the AII induced $^{45}Ca^{2+}$ efflux from rat aortic smooth muscle cells.

Ex vivo functional activity in rabbit aorta.

Functional activity is measured by the ability of the multi-binding compound to antagonize the functional contractile response to angiotensin II in a dose-dependent manner to provide a pA_2 value in a manner similar to that set forth in A. T. Chiu et al.[47].

In vivo **model**-Lack of effect on blood pressure in conscious normotensive rats.

The effect on pressor response to the multi-binding compound is tested by methods similar to that set forth in P.C. Wong et al.[48].

In vivo **model**- hypertensive rats

The effect of the multi-binding compound on blood pressure in the renal-artery ligated rat, a high renin hypertensive rat model, is tested using methods similar to that set forth in P.C. Wong et al.[49].

EXAMPLE 9

β2-adrenergic Agonist Multivalomers for Airway Disease.

β2-adrenergic agonists are used in the clinic for the treatment of acute symptoms of asthma, chronic asthma and chronic obstructive airway disease. For example, salmeterol acts within 60-90 minutes of exposure. β2-adrenergic agonists appear to be generally administered by inhalation or less frequently orally. In general, the route of administration also significantly effects the onset of action with inhalation being significantly more rapid than oral dosing.

The current compounds are distinguished by their onset of action. This defines whether they are rapid onset such terbutaline (1-5 minutes) or whether they have a slower onset. The medicines that are prescribed are further distinguished by their duration of action. This defines whether they have a shorter duration of action e.g. albuterol (3-4 hours) or fenterol (12-14 hrs).

Formoterol, a selective β2-adrenergic agonist, produces ED-proportional bronchodilation, which persists for up to 12 hours, in patients with reversible obstructive respiratory disease. Bronchodilation is significant within minutes of inhalation, maximal within 2 hours, and at therapeutic doses is equivalent to that

5   produced by standard doses of traditional β2-adrenergic agonist (Robert R. Ruffolo et al.[34]).

The following references describe the clinical pharmacology of a number of β2-adrenergic agonists currently used in human medicine (Louis Philippe Boulet[35], Claes-Goran A. Lofdahl[36] and Rebecca A. Bartow and Rex N. Brogden[37]).

10   The two categories (classified by their pharmacological profile that defines their clinical utility) of β_2-adrenergic agonist are set forth in Figure 47.
i) the rapid onset/ shorter acting drugs. Specifically multivalomers are derived from the monovalomeric marketed drugs albuterol and terbutaline. Multivalomers are also derived from other β2-adrenergic agonists that are in clinical use e.g

15   bambuterol (prodrug), bitolterol, carbuterol, clenbuterol, colterol, fenoterol, indenolol, mabuterol, metaproterenol pirbuterol procaterol, reproterol, tulobuterol.
ii) slower onset/prolonged duration of action. Specifically multivalomers are derived from formoterol and salmeterol.

It is contemplated that both of these qualities of rapid onset of action and

20   prolonged duration may be introduced into one molecule. It is also contemplated that the multivalomeric ligand could incorporate the elements of the rapid onset pharmacophore and elements of the prolonged duration of action pharmacophore in the one molecule

The following are some of the monovalomer drugs that used to design the

25   pharmacophoric building blocks to allow multivalomer construction.

Albuterol (VENTOLIN) is a selective β_2-adrenergic agonist with pharmacological properties and therapeutic indications similar to those of terbutaline. It is administered either by inhalation or orally for the symptomatic relief of bronchospasm. When administered by inhalation, it produces significant

bronchodilation within 15 minutes, and effects are demonstrable for 3 to 4 hours. The cardiovascular effects of albuterol are considerably weaker than those of isoproterenol when doses that produce comparable bronchodilatation are administered by inhalation

5   Bitolterol is a β_2-adrenergic agonist in which the hydroxyl groups in the catechol moiety are protected by esterification with 4-methylbenzoate. Esterases in the lung and other tissues hydrolyze this prodrug to the active form, colterol, or terbutylnorepinephrine. Results of animal studies have suggested that these esterases are present in higher concentration in lung than in tissues such as the

10   heart. The duration of effect of bitolterol after inhalation ranges from 3 to 6 hours.

Fenoterol (BEROTEC) is a β_2-selective adrenergic receptor agonist. It has a rapid onset and its effect is typically sustained for 2 to 3 hours.

Formoterol (FORADIL) is a long-acting β_2-selective adrenergic receptor agonist. Significant bronchodilation occurs within minutes and persists for up to 12

15   hours. Its major advantage over many other β_2-selective agonists is this prolonged duration of action, which may be particularly advantageous in settings such as nocturnal asthma.

Metaproterenol (ALUPENT) is used for the long-term treatment of obstructive airway diseases and for treatments of acute bronchospasm.

20   Metaproterenol is considered to be β_2-selective, although it probably is less selective than albuterol or terbutaline. Effects occur within minutes of inhalation and persist for several hours. After oral administration, onset of action is slower, but effects last 3 to 4 hours.

Pirbuterol is a relatively selective β_2 agonist. It is structurally identical to

25   albuterol except for the substitution of a pyridine ring for the benzene ring.

Procaterol (MASCACIN) is a β_2-selective adrenergic receptor agonist. It has a rapid onset of action, which is sustained for about 5 hours.

Salmeterol (SEREVENT) is a β_2-selective adrenergic receptor agonist with a prolonged duration of action, of around 12 hours. However, it has a relatively slow

onset of action after inhalation, so is not suitable alone for prompt relief of

breakthrough attacks of bronchospasm. *In vitro*, salmeterol exerts 'reassertion'

relaxation of airways smooth muscle. Reassertion relaxation refers to the capacity

of salmeterol to cause repeated functional relaxation of induced contraction when

5   airway smooth muscle is intermittently exposed to, then washed free from,

β_2-adrenoceptor antagonists such as sotalol. The mechanism(s) underlying

reassertion relaxation may relate to high affinity binding of the long aliphatic side

chain of salmeterol to an accessory site, distinct from the agonist recognition site,

in or near the β2-adrenoceptor (exosite binding hypothesis).

10   Terbutaline is a β_2-selective bronchodilator. It is effective when taken orally,

subcutaneously, or by inhalation. Effects are observed rapidly after inhalation or

parenteral administration. After inhalation its action may persist for 3 to 6 hours.

With oral administration, the onset of effect may be delayed for 1 to 2 hours. It is

also used for the long-term treatment of obstructive airway diseases and for

15   treatment of acute bronchospasm. Furthermore, it is available for parenteral use

for the emergency treatment of status asthmaticus.

a) Design of β_2-adrenergic ligand multivalomers.

Figure 48 sets forth the preferred sites for attachment of a ligand to albuterol

20   for the generation of multivalomers. M represents the site for attachment. Figures

49 to 54 set forth the various valencies and orientations of the ligands. Figures 49

and 54 illustrate mixed multivalomers derived from different β_2-agonists.

1)   Synthesis of Bivalent Analogs of Salmeterol (Figure 55)

25   With reference to Figure 55, compounds (1) (n = 1; 305 mg; 1.12 mmole)

and (2) (200 mg; 0.5 mmole) were dissolved in 6 mL of N,N-dimethylformamide

(DMF) followed by addition of HOBt (144 mg) and DIPEA (0.22 mL). To this

stirred solution was added PyBOP (554 mg) as a solid. The final mixture was

stirred at ambient temperature for 24 hours. The reaction mixture was diluted with

brine (100 mL), and extracted with EtOAc (150 mL). The organic phase was
washed with 0.1 M HCl, 0.1 M NaOH, and brine, followed by drying with
MgSO₄. Evaporation of the organic solution afforded a pale yellow oily residue,
which was purified by flash silica column chromatography (1/1 EtOAc/hexanes to

5   0.1/1/1 MeOH/EtOAc/hexanes):Rf of product (3) = 0.68 in 0.1/1/1
MeOH/EtOAc/hexanes.

Compound (3) (358 mg; 0.4 mmole) was added slowly to a stirred suspension
of LiAlH₄ (120 mg; 3.2 mmole) in THF (40 mL) cooled by an ice bath. The
reaction mixture was slowly warmed to room temperature (30 min), and refluxed at

10 80°C for 4 hours. After cooling the mixture in an ice bath, 15% NaOH (0.5 mL)
was added to quench the reaction, followed by stirring 30 min. The reaction
mixture was filtered, and the solid residue was washed with 10% MeOH/THF (50
mL). Filtrates were combined, and evaporated *in vacuo*, yielding a pale yellow
oily residue. It was purified by flash silica column chromatography (2%

15 MeOH/CH₂Cl₂ to 2% i-PrNH₂/10% MeOH/CH₂Cl₂): Rf of product (4) (yield 179
mg) = 0.47 in 2% i-PrNH₂/10% MeOH/CH₂Cl₂.

Compound (4) (170 mg; 0.2 mmole) was dissolved in EtOH (50 mL)
containing 10% Pd/C (100 mg). The mixture was degassed, and saturated with H₂
gas. After stirring the mixture under H2 atmosphere (1 atm) for 24 hours at

20 ambient temperature, the catalyst was filtered, and washed with EtOH (50 mL).
Filtrates were combined, and evaporated, yielding a colorless oily residue. The
product (5) was dissolved in 30% MeCN/water (containing 0.5% TFA), and
purified by reversed-phase HPLC: 116 mg was obtained.

H1-NMR of 5 (CD₃OD, 299.96 MHz): δ = (ppm) 7.35-7.34 (d, J = 2.1 Hz; 2H),

25 7.27-7.11 (7H), 6.80-6.77 (d, J = 8.1 Hz; 2H), 4.84 (m, 2H), 4.65 (s, 4H),
3.67-3.46 (m, 1H), 3.44-3.37 (m, 6H), 3.15-2.99 (m, 8H), 2.75-2.61 (m, 2H),
1.83-1.68 (m, 6H), 1.59-1.50 (broad s, 4H), 1.48-1.40 (broad s, 8H);
Electrospray Mass Spectrum (C₄₀H₆₀N₂O₈): calculated., 696.8, observed. 697.7 [M
+ H]+.

2)   Synthesis of Bivalent Analogs of Salmeterol (Figure 56)

With reference to Figure 56, compounds (1) (n = 3; 450 mg; 1.65 mmole) and (6) (400 mg; 0.6 mmole) were dissolved in 10 mL of DMF followed by addition of HOBt (208 mg) and DIPEA (0.34 mL). To this stirred solution was
5   added PyBOP (802 mg) as solid. The final mixture was stirred at ambient temperature for 24 hours. The reaction mixture was diluted with brine (100 mL), and extracted with EtOAc (150 mL). The organic phase was washed with 0.1 M HCl, 1 M NaOH, and brine, followed by drying with $MgSO_4$. Evaporation of the organic solution afforded a pale yellow oily residue (720 mg): Rf of product (7) =
10   0.68 in 20% $MeOH/CH_2Cl_2$. H1-NMR (CD_3OD, 300 MHz): δ = (ppm).

Compound (7) (720 mg; 0.61 mmole) was added slowly to a stirred suspension of $LiAlH_4$ (265 mg; 7.0 mmole) in THF (50 mL) cooled by an ice bath. The reaction mixture was slowly warmed to room temperature (30 min), and refluxed at 80°C for 4 hours. After cooling of the mixture with ice bath, 15%
15   NaOH (1.0 mL) was added to quench the reaction, followed by stirring 30 min. The reaction mixture was filtered, and the solid residue was washed with 10% MeOH/THF (50 mL). Filtrates were combined, and evaporated in vacuo, yielding a pale yellow oily residue. It was purified by flash silica column chromatography (2% MeOH/CH2Cl2 to 2% i-PrNH2/10% MeOH/CH2Cl2) to afford product (8)
20   (yield 370 mg).

Compound (8) (370 mg; 0.32 mmole) was dissolved in EtOH (50 mL) containing 10% Pd/C (150 mg). The mixture was degassed, and saturated with H_2 gas. After stirring the mixture under H_2 atmosphere (1 atm) for 24 hours at ambient temperature, the catalyst was filtered, and washed with EtOH (50 mL).
25   Filtrates were combined, and evaporated, yielding a colorless oily residue. The crude product (9) was dissolved in 30% MeCN/water (containing 0.5% TFA), and purified by reversed-phase HPLC.

H1-NMR of compound (9) (CD_3OD, 299.96 MHz): δ = (ppm) 7.35-7.34 (d, J = 2.1 Hz; 2H), 7.18-7.15 (dd , J = 2.1, 7.8 Hz; 2H), 7.08-7.05 (d, J = 8.4 Hz; 4H),

6.81-6.77 (m, 6H), 4.84-4.82 (m, 2H), 4.65 (s, 4H), 3.94-3.89 (t, J = 6.6 Hz; 4H), 3.45-3.4 (quin, 8H), 3.12-3.11 (dd, 4H), 3.09-3.01 (dd, 4H), 2.61-2.56 (t, 4H), 1.84-1.69 (m, 12H), 1.60-1.55 (m, 4H), 1.5-1.29 (m, 20H).

Electrospray Mass Spectrum ($C_{58}H_{88}N_2O_{10}$): calculated. 973.2, observed. 973.8 [M]+, 487.6 [M + 2H]2+.

3)   Synthesis of Bivalent Analogs of Salmeterol (Figure 57)

With reference to Figure 57, compounds (10) (n = 1; 20 mg; 0.038mmole) and 1,6-hexanedioic acid (2.6 mg; 0.018 mmole) were dissolved in 1.5 mL of DMF followed by addition of HOBt (5.4 mg) and DIPEA (0.009 mL). To this stirred solution was added PyBOP (21 mg) as solid. The final mixture was stirred at ambient temperature for 24 hours. The reaction mixture was diluted with brine (30 mL), and extracted with EtOAc (50 mL). The organic phase was washed with 0.1 M HCl, 0.1 M NaOH, and brine, followed by drying with MgSO4. Evaporation of the organic solution afforded a pale yellow oily residue. The residue was dissolved in CH_2Cl_2 (1 mL), cooled in ice bath, followed by addition of CH_2Cl_2/TFA (1:1; 1 mL). After stirring 3 hours at 0°C, the mixture was evaporated *in vacuo*, yielding product (11) as a pale yellow oil. It was purified by reversed-phase HPLC.

H1-NMR of product (11) (CD_3OD, 299.96 MHz): δ = (ppm) 7.43-7.40 (d, J = 8.1 Hz; 4H), 7.33-7.22 (d, J = 2.1 Hz; 2H), 7.17-7.10 (m, 6H), 6.79-6.77 (d, J = 8.1 Hz; 2H), 4.65 (m, 2H), 4.58 (s, 4H), 3.45-3.40 (m, 8H), 3.10-3.07 (dd, 4H), 3.03-2.98 (dd, 4H), 2.61-2.56 (t, J = 7.5 Hz; 4H), 2.61-2.56 (t, J = 7.5 Hz; 4H), 2.36-2.30 (t, J = 7.5 Hz; 4H).

4)   Synthesis of Bivalent Analogs of Alprenolol (Figure 58)

With reference to Figure 58, compounds (12) (450 mg; 1.05 mmole) and terphthalic acid (88 mg; 0.53 mmole) were dissolved in 5 mL of DMF followed by addition of HOBt (150 mg) and DIPEA (0.19 mL). To this stirred solution was

added PyBOP (579 mg) as solid. The final mixture was stirred at ambient temperature for 24 hours. The reaction mixture was diluted with brine (100 mL), and extracted with CH_2Cl_2 (150 mL). The organic phase was washed with 0.1 M HCl, 0.1 M NaOH, and brine, followed by drying with $MgSO_4$. Evaporation of the organic solution afforded a pale yellow oily residue, which was then purified by flash silica column chromatography: 4/1 to 1/1 hexanes/EtOAc; Rf of product (13) (472 mg) = 0.52 in 3/1 hexanes/EtOAc.

Compound (13) (102 mg; 0.1 mmole) was dissolved in CH_2Cl_2 (2 mL), cooled in ice bath, followed by addition of CH_2Cl_2/TFA (1:1; 4 mL). After stirring 3 hours at 0°C, the mixture was evaporated *in vacuo*, yielding product (14) as a pale yellow oil. It was purified by reversed-phase HPLC.

H1-NMR of product (14) (CD_3OD, 299.96 MHz): δ = (ppm) 7.88 (s, 4H), 7.18-7.11 (m, 4H), 6.91-6.89 (m, 4H), 4.30-4.24 (m, 2H), 4.09-4.04 (dd, 2H), 4.00-3.95 (dd, 2H), 3.58-3.53 (t, J = 7.8 Hz; 4H), 3.50-3.42 (hept, 2H), 3.31-3.30 (dd, 2H), 3.23-3.15 (dd, 2H), 2.78-2.71 (m, 8H), 2.63-2.60 (t, J = 7.2 Hz; 4H),1.92-1.83 (quin, J = 7.2 Hz; 4H), 1.37-1.34 (double d, 12H); Electrospray Mass Spectrum ($C_{42}H_{62}N_4O_6S_2$): calculated. 782.9, obsserved. 783.5 [M]+.

Compound (13) (370 mg; 0.38 mmole) was added slowly to a stirred suspension of $LiAlH_4$ (100 mg; 2.64 mmole) in THF (20 mL) cooled by an ice bath. The reaction mixture was slowly warmed to room temperature (30 min), and refluxed at 80°C for 4 hours. After cooling of the mixture with ice bath, 15% NaOH (0.5 mL) was added to quench the reaction, followed by stirring for 30 min. The reaction mixture was filtered, and the solid residue was washed with 10% MeOH/THF (50 mL). Filtrates were combined, and evaporated *in vacuo*, yielding a pale yellow oily residue. It was purified by flash silica column chromatography (5% $MeOH/CH_2Cl_2$ to 2% i-$PrNH_2$/10% $MeOH/CH_2Cl_2$) to afford product (15). The product was then dried and dissolved in CH_2Cl^2 (2 mL), cooled in ice bath, followed by addition of CH_2Cl_2/TFA (1:1; 4 mL). After stirring 3 hours at 0°C,

the mixture was evaporated *in vacuo*, yielding product (16) as a pale yellow oil. It was purified by reversed-phase HPLC.

H1-NMR of product (16) (CD$_3$OD, 299.96 MHz):δ = (ppm) 7.37 (s, 4H), 7.14-7.08 (m, 4H), 6.85-6.82 (m, 4H), 4.09 (s, 4H), 4.0-3.82 (m, 4H), 3.06-3.01(t, 4H), 2.67-2.63 (t, 4H), 2.37-2.33 (t, 4H), 1.69-1.64 (quin, 4H), 1.21-1.12 (m, 12H).

b) Evaluation of β_2- adrenergic Multivalomers.

These multivalomers are evaluated for their ability to deliver a selective adrenoreceptor agonist. The pharmacological properties of potency, onset of action, duration of action, on and off rates are measured for these multivalomers at the β_2 adrenergic receptor.

The multivalomers are tested by *in vitro* radioligand binding to evaluate the potency of the multivalomers at the β_2 adrenergic receptor.

The affinity of these multivalomers for the β_2 adrenergic receptor is carried out by a method similar to that set forth in Kobilka et al.[50]. Preliminary radioligand binding assays are used to determine the affinity of the ligand for the human and animal β_2 adrenergic receptors. This provides the pKi for the multivalomer ligands at these receptors. Preferably the multivalomer will show a 1-10 nm affinity at the β_2 adrenergic receptor but show a sub-micromolar affinity at the other adrenergic receptors. See also Richard A. Dixon et al.[51] and Stuart Green et al.[52] for methods using radioligand binding studies at the β_2 -adrenergic receptor and procedures to determine the functional response of multivalomers at this receptor.

The functional activity of these multivalomers is evaluated by a method similar to that set forth in Stuart Green et al.[53]; Sturat Green and Stephen Liggett[54]; Liggett et al.[55]; and Karen McCrea and Stephen Hill[56].

These multivalomers are then shown to provide functional responses in tissues that are known to be surrogates of the clinic. Initially this is carried out

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using animal tissues. Compounds of a suitable profile also have their activity confirmed in human tissue.

   These *ex vivo* animal studies are carried out using guinea pig pulmonary tissue according to a method similar to the protocol set forth in Bertil Waldeck and

5 Erik Widmark[57]. The relaxation of the carbachol-contracted trachea, increase in the force of contraction of the papillary muscle and depression of subtetanic contractions of the soleus muscle are studied (A. Bergendal et al.[58] and B. Waldeck et al.[61]). The effects measured are a) relaxation of the tracheal smooth muscle (mostly β_2); b) depression of subtetanic contractions of the soleus muscle (β_2); and

10 c) increase in the force of the papillary muscle of the left ventricle (β_1). .

   The compounds are tested in human tissues by methods similar to those set forth in Anthony T. Nials et al.[59 and 60].

   The compounds are also tested *in vivo* by the method set forth in Brittain et al.[62].

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EXAMPLE A1
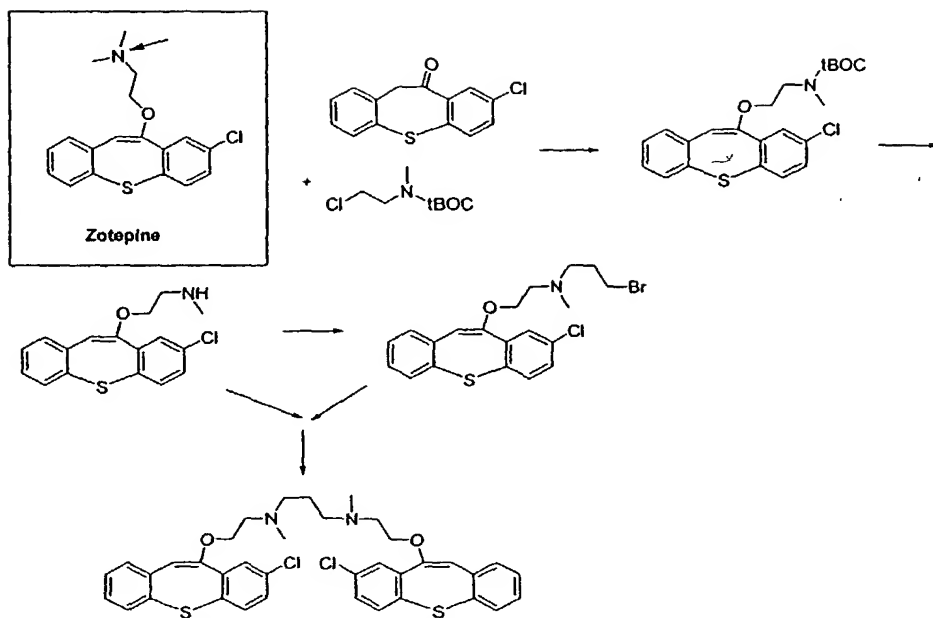
Sodium hydride (5 mmol) is added to a solution of 8-chlorodibenzo[b,f]thiepin-10(11H)-one, prepared as described in Chem. Pharm. Bull., 1975, 23, 2223, (5 mmol) in

5   toluene (25 mL) at 65°. After 1 hour, 2-[N-(tert-butoxycarbonyl-N-methylamino)ethyl chloride, prepared as described in J. Med. Chem., 1998, 41, 5429, (10 mmol) is added. The progress of the reaction is monitored by tlc. When it is complete, the solution is cooled and it is added to water. The organic phase is washed, dried and evaporated, and the residue is chromatographed to afford 8-chloro-10-[2-(N-methyl-N-tert-

10   butoxycarbonylamino)ethoxy]dibenzo[b,f]thiepin.

The above compound (1 mmol) is dissolved in CH₂Cl₂ (10 mL) and TFA (5 mL) is added. The progress of the reaction is monitored by tlc. When it is complete, the solvents are removed under vacuum and the residue is dissolved in CH₂Cl₂. The solution washed with dilute NaOH, then the organic phase is washed, dried and evaporated, and the residue

15   is chromatographed to afford 8-chloro-10-[2-(N-methylamino)ethoxy]dibenzo[b,f]thiepin.

8-Chloro-10-[2-(N-methylamino)ethoxy]dibenzo[b,f]thiepin (2 mmol) is dissolved in DMF (10 mL) and K₂CO₃ (0.5g), KI (50 mg) and 1,3-dibromopropane (2 mmol) are added. The mixture is heated to 60° and the progress of the reaction is monitored by tlc. When it is complete, the solution is cooled and it is added to water. The aqueous solution is extracted

20   with EtOAc. The organic phase is washed, dried and evaporated, and the residue is

chromatographed to afford 10-[2-N-(3-bromopropyl)(N-methylamino)ethoxy]-8-chlorodibenzo[b,f]thiepin.

8-Chloro-10-[2-(N-methylamino)ethoxy]dibenzo[b,f]thiepin (1 mmol) and 10-[2-N-(3-bromopropyl)(N-methylamino)ethoxy]-8-chlorodibenzo[b,f]thiepin (1 mmol) are heated at reflux in EtOH (15 mL). The progress of the reaction is monitored by tlc. When it is complete, the solution is added to water and extracted with CH_2Cl_2. The organic phase is washed, dried and evaporated, and the residue is chromatographed to afford the compound.

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EXAMPLE A2

Zotepine
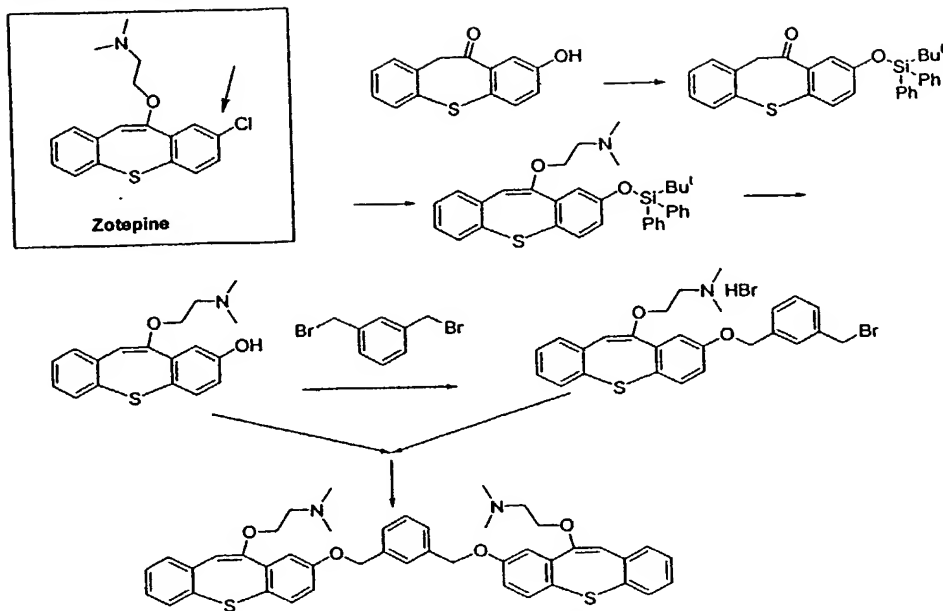
8-Hydroxydibenzo[b,f]thiepin-10(11H)-one, prepared as described in Coll. Czech.
Chem. Commun., 1975, 23, 2223, (5 mmol) is dissolved in DMF (50 mL) and imidazole
5   (10 mmol) and tert-butylchlorodiphenylsilane (6 mmol) are added. The progress of the
reaction is monitored by tlc. When it is complete, the solution is added to water and
extracted with CH_2Cl_2. The organic phase is washed, dried and evaporated, and the residue
is chromatographed to afford 8-(tert-butyldiphenylsilyloxy)dibenzo[b,f]thiepin-10(11H)-
one.

10   Sodium hydride (5 mmol) is added to a solution of the compound above (5 mmol) in
toluene (25 mL) at 65°. After 1 hour, 2-chloro-N,N-dimethylethylamine (10 mmol) is
added. The progress of the reaction is monitored by tlc. When it is complete, the solution is
cooled and it is added to water. The organic phase is washed, dried and evaporated, and the
residue is chromatographed to afford 8-(tert-butyldiphenylsilyloxy)-10-(2-N,N-
15   dimethylaminoethoxy)dibenzo[b,f]thiepin.

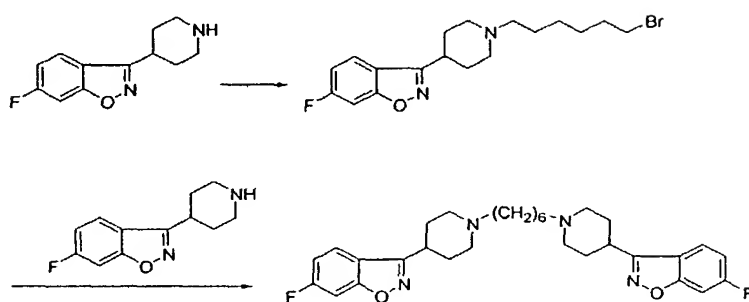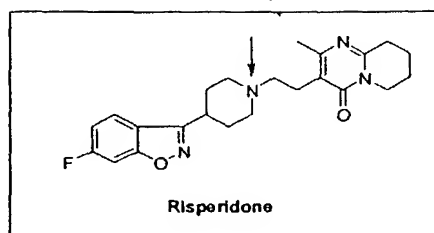The above compound (1 mmol) is dissolved in THF (10 mL) and 1M Bu_4NF in THF
(20 mL) is added. The progress of the reaction is monitored by tlc. When it is complete, the
solution is added to water and extracted with CH_2Cl_2. The organic phase is washed, dried
and evaporated, and the residue is chromatographed to afford 8-hydroxy-10-(2-N,N-
20   dimethylaminoethoxy)dibenzo[b,f]thiepin.

The above compound (2 mmol) is dissolved in DMF (20 mL) and 1,3-di(bromomethyl)benzene (2 mmol) and K$_2$CO$_3$ (0.5g) are added. The progress of the reaction is monitored by tlc. When it is complete, the solution is added to water and extracted with CH$_2$Cl$_2$. The organic phase is washed, dried and evaporated, and the residue is chromatographed to afford afford 8-(3-bromomethylbenzyloxy)-10-(2-N,N-dimethylaminoethoxy)dibenzo[b,f]thiepin stored as the hydrobromide salt.

8-Hydroxy-10-(2-N,N-dimethylaminoethoxy)dibenzo[b,f]thiepin (1 mmol) and 8-(3-bromomethylbenzyloxy)-10-(2-N,N-dimethylaminoethoxy)dibenzo[b,f]thiepin (1 mmol) are dissolved in DMF (15 mL) and K$_2$CO$_3$ (0.5 g) is added. The progress of the reaction is monitored by tlc. When it is complete, the solution is added to water and extracted with CH$_2$Cl$_2$. The organic phase is washed, dried and evaporated, and the residue is chromatographed to afford the desired dimeric ether.

EXAMPLE A3
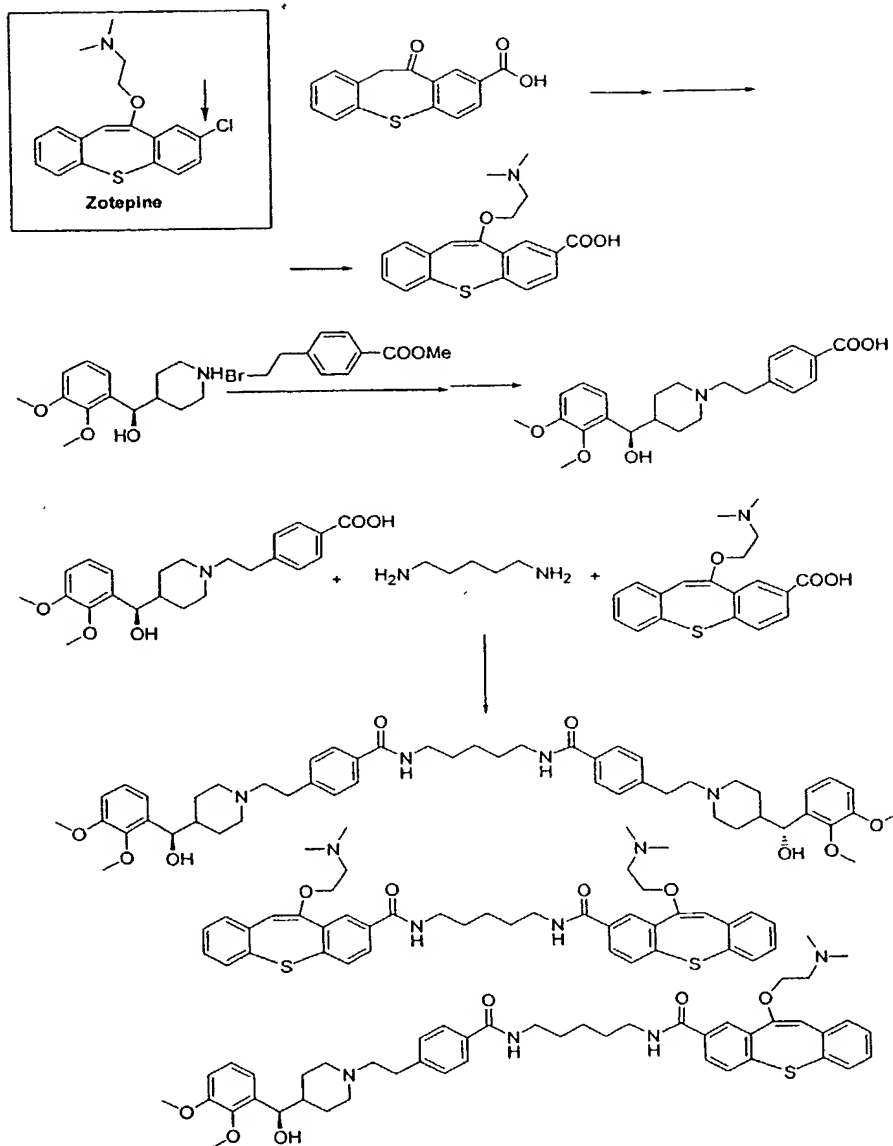
Sodium hydride (10 mmol) is added to a solution of 8-carboxydibenzo[b,f]thiepin-10(11H)-one (5 mmol) in toluene (25 mL) at 65°. After 1 hour, 2-chloro-N,N-dimethylethylamine (10 mmol) is added. The progress of the reaction is monitored by tlc. When it is complete, the solution is cooled and it is added to water. The organic phase is washed, dried and evaporated, and the residue is chromatographed to afford compound 2-dimethylaminoethyl 10-[2-(N,N-dimethylamino)ethoxy]dibenzo[b,f]thiepin-8-carboxylate.

The above compound (1 mmol) is dissolved in THF (10 mL) and a solution of LiOH (2 mmol) in water (10 mL) is added. The progress of the reaction is monitored by tlc. When it is complete, the solution is added to water; the pH is adjusted to 7 by addition of dilute HCl, and the solution is extracted with CH_2Cl_2. The organic phase is washed, dried and

5   evaporated, and the residue is chromatographed to afford into 10-[2-(N,N-dimethylamino)ethoxy]dibenzo[b,f]thiepin 8-carboxylic acid.

(R)-α-(2,3-dimethoxyphenyl)-4-piperidinemethanol, prepared as described in EP 531410, (2 mmol) is dissolved in DMF (10 mL) with K_2CO_3 (0.5g), KI (50 mg) and 2-(4-carbomethoxyphenyl)ethyl bromide, prepared as described in J. Med. Chem., 1993, 36,

10   1880 (2 mmol) are added. The mixture is heated to 60° and the progress of the reaction is monitored by tlc. When it is complete, the solution is cooled and it is added to water. The aqueous solution is extracted with EtOAc. The organic phase is washed, dried and evaporated, and the residue is chromatographed to afford (R)-α-(2,3-dimethoxyphenyl)-1-[2-(4-carbomethoxyphenyl)ethyl]-4-piperidinemethanol.

15   The above compound (1 mmol) is dissolved in THF (10 mL) and a solution of LiOH (2 mmol) in water (10 mL) is added. The progress of the reaction is monitored by tlc. When it is complete, the solution is added to water; the pH is adjusted to 7 by addition of dilute HCl, and the solution is extracted with CH_2Cl_2. The organic phase is washed, dried and evaporated, and the residue is chromatographed to afford (R)-α-(2,3-dimethoxyphenyl) 1-

20   [2-(4-carboxyphenyl)ethyl]-4-piperidinemethanol.

10-[2-(N,N-dimethylamino)ethoxy]dibenzo[b,f]thiepin 8-carboxylic acid (1 mmol), (R)-α-(2,3-dimethoxyphenyl) 1-[2-(4-carboxyphenyl)ethyl]-4-piperidinemethanol (1 mmol) and 1,5-pentanediamine (0.5 mmol) and dicyclohexylcarbodiimide (2 mmol) are dissolved in DMF (10 mL). The progress of the reaction is monitored by tlc. When it is

25   complete, the solution is added to water and extracted with CH_2Cl_2. The organic phase is washed, dried and evaporated, and the residue is chromatographed to afford the desired dimeric amides.
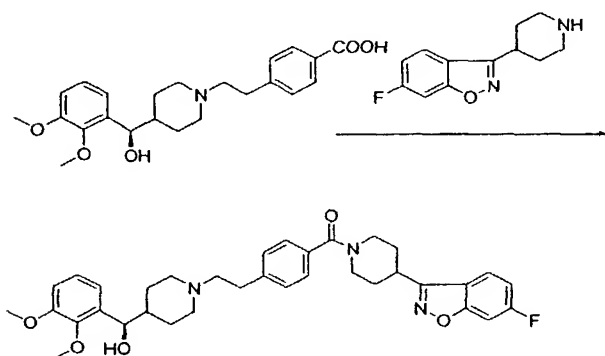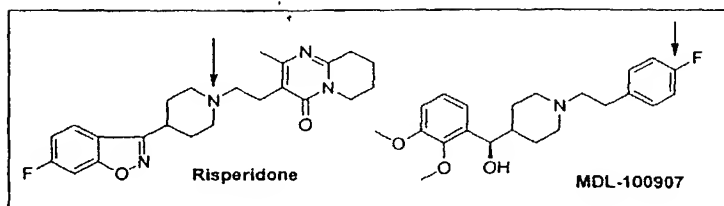
EXAMPLE A4

Risperidone

3-(4-Piperidinyl)-6-fluorobenz[d]isoxazole, prepared as described in J. Med. Chem.,
1985, 28, 761, (2 mmol) is dissolved in DMF (10 mL) and K₂CO₃ (0.5g), KI (50 mg) and

5   1,6-dibromohexane (2 mmol) are added. The mixture is heated to 60° and the progress of
the reaction is monitored by tlc. When it is complete, the solution is cooled and it is added
to water. The aqueous solution is extracted with EtOAc. The organic phase is washed,
dried and evaporated, and the residue is chromatographed to afford 3-[4-[1-(6-
bromohexyl)piperidinyl]]-6-fluorobenz[d]isoxazole.

10   3-[4-[1-(6-Bromohexyl)piperidinyl]]-6-fluorobenz[d]isoxazole (1 mmol) and 3-(4-
piperidinyl)-6-fluorobenz[d]isoxazole (1 mmol) are heated at reflux in EtOH (20 mL)
containing diisopropylethylamine (5 mmol) The progress of the reaction is monitored by
tlc. When it is complete, the cooled solution is added to water and extracted with CH₂Cl₂.
The organic phase is washed, dried and evaporated, and the residue is chromatographed to

15   afford the desired dimeric compound.

EXAMPLE A5

Risperidone   MDL-100907


3-(4-Piperidinyl)-6-fluorobenz[d]isoxazole, prepared as described in J. Med. Chem.,
1985, 28, 761, (R)-α-(2,3-dimethoxyphenyl) 1-[2-(4-carboxyphenyl)ethyl]-4-

5   piperidinemethanol (1 mmol) (prepared in Example A3) and dicyclohexylcarbodiimide (1
mmol) are dissolved in DMF (20 mL). The progress of the reaction is monitored by tlc.
When it is complete, the solution is added to water and extracted with CH_2Cl_2. The organic
phase is washed, dried and evaporated, and the residue is chromatographed to afford the
desired dimeric compound.

EXAMPLE A6
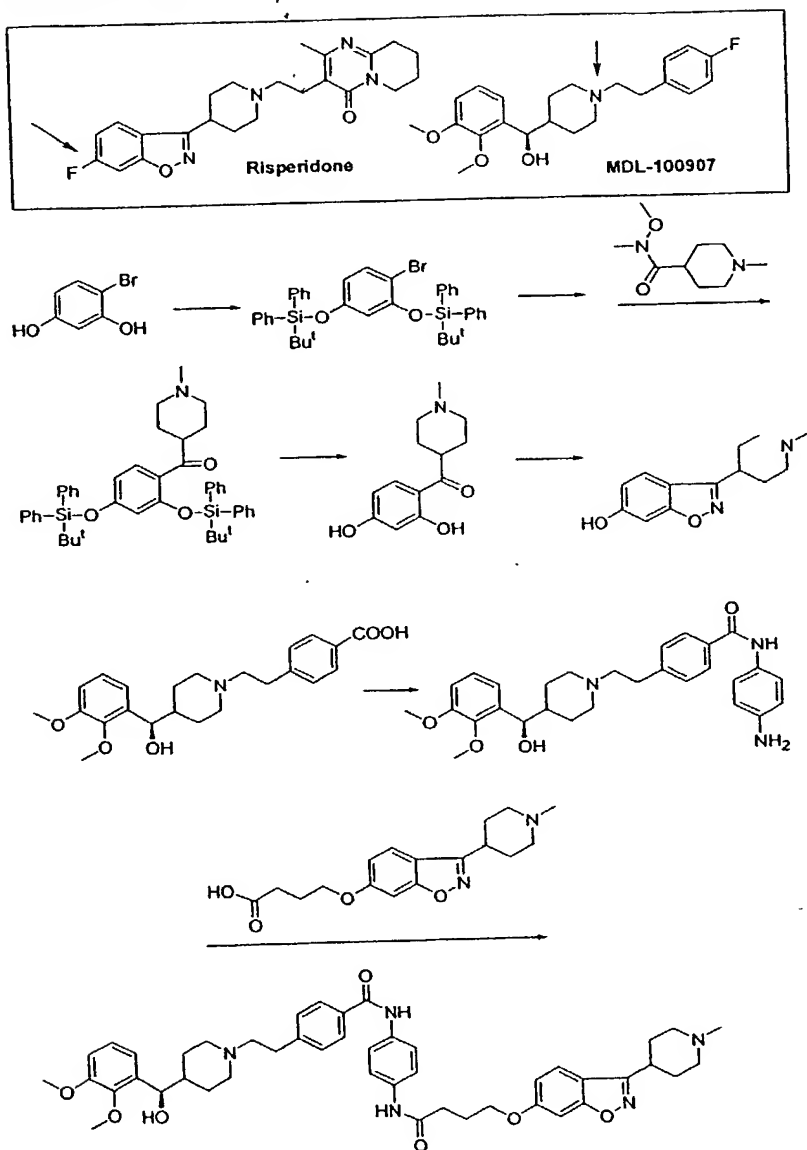
4-Bromo-1,3-dihydroxybenzene (5 mmol) is dissolved in DMF (50 mL) and imidazole

(10 mmol) and tert-butylchlorodiphenylsilane (12 mmol) are added. The progress of the

5   reaction is monitored by tlc. When it is complete, the solution is added to water and

extracted with CH_2Cl_2. The organic phase is washed, dried and evaporated, and the residue

is chromatographed to afford 4-bromo-1,3-di(tert-butyldiphenylsilyloxy)benzene

The above compound (2 mmol) is dissolved in THF (20 mL) under an inert

atmosphere, and 1N n-BuLi in hexane (2 mL) is added. After 1 hour, the solution is cooled

to -78° and a solution of 4-(N-methoxy-N-methylcarbonyl)-N-methylpiperidine, prepared as described in WO9850346, (2 mmol) in THF (10 mL) is added. The progress of the reaction is monitored by tlc. When it is complete, the solution is added to water and extracted with CH_2Cl_2. The organic phase is washed, dried and evaporated, and the residue

5   is chromatographed to afford 4-[2,4-di(tert-butyldiphenylsilyloxy)benzoyl]-N-methylpiperidine.

The above compound (1 mmol) is dissolved in THF (10 mL) and 1M Bu_4NF in THF (20 mL) is added. The progress of the reaction is monitored by tlc. When it is complete, the solution is added to water and extracted with CH_2Cl_2. The organic phase is washed, dried

10   and evaporated, and the residue is chromatographed to afford 4-(2,4-dihydroxyoxybenzoyl)-N-methylpiperidine.

To a solution of hydroxylamine hydrochloride (5 mmol) in EtOH (25 mL) is added 1M NaOH (5 mL) and the above compound (1 mmol). The solution is heated at reflux and the progress of the reaction is monitored by tlc. When it is complete, the cooled solution is

15   added to water and extracted with CH_2Cl_2. The organic phase is washed, dried and evaporated, and the residue is chromatographed to afford 6-(3-carboxypropoxy)-3-[(4-(N-methylpiperidinylbenz[d]isoxazole.

The acid prepared in Example A3 (1 mmol) is dissolved in DMF (20 mL) and

20   dicyclohexylcarbodiimide (1 mmol) and 1,4-diaminobenzene (1 mmol) are added. The progress of the reaction is monitored by tlc. When it is complete, the solution is added to water and extracted with CH_2Cl_2. The organic phase is washed, dried and evaporated, and the residue is chromatographed to afford the 4-aminophenyl amide.

6-(3-Carboxypropoxy)-3-[(4-(N-methylpiperidinylbenz[d]isoxazole (prepared from the

25   isoxazole above by technique described in previous examples) the 4-aminophenyl amide (1 mmol) and dicyclohexylcarbodiimide (1 mmol) are dissolved in DMF. The progress of the reaction is monitored by tlc. When it is complete, the solution is added to water and extracted with CH_2Cl_2. The organic phase is washed, dried and evaporated, and the residue is chromatographed to afford the desired dimeric amide.

30

EXAMPLE A7

3-(4-Piperidinyl)-6-fluorobenz[d]isoxazole, prepared as described in J. Med. Chem., 1985, 28, 761, (2 mmol) is dissolved in DMF (10 mL) and K_2CO_3 (0.5g), KI (50 mg) and

5   1,6-dibromohexane (2 mmol) are added. The mixture is heated to 60° and the progress of the reaction is monitored by tlc. When it is complete, the solution is cooled and it is added to water. The aqueous solution is extracted with EtOAc. The organic phase is washed, dried and evaporated, and the residue is chromatographed to afford 3-[4-[1-(6-bromohexyl)piperidinyl]]-6-fluorobenz[d]isoxazole.

10   3-Hydroxy-2-methoxybromobenzene, prepared as described in Tetrahedron Letters, 1984, 36, 3955, (5 mmol) is dissolved in DMF (50 mL) and imidazole (10 mmol) and tert-
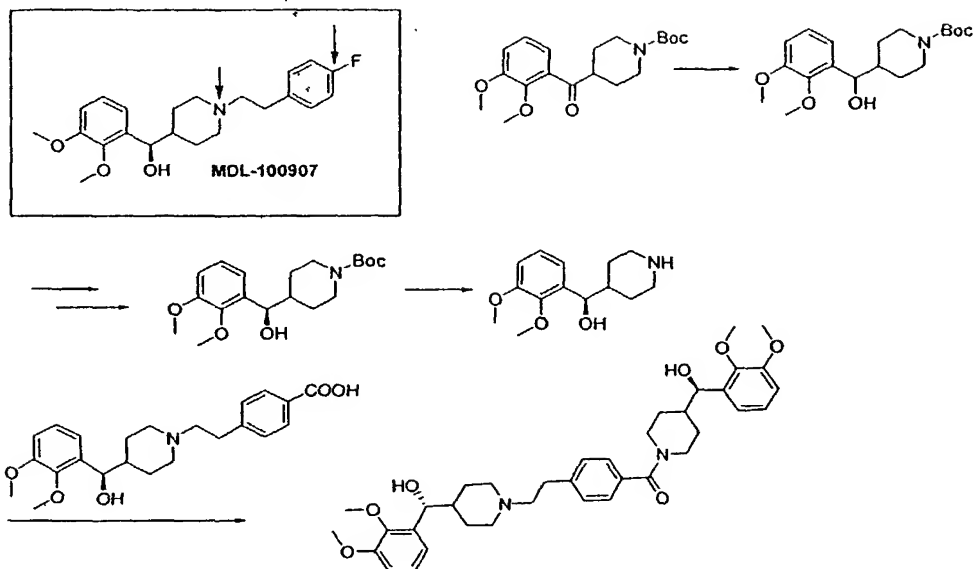
butylchlorodiphenylsilane (6 mmol) are added. The progress of the reaction is monitored by tlc. When it is complete, the solution is added to water and extracted with CH_2Cl_2. The organic phase is washed, dried and evaporated, and the residue is chromatographed to afford 3-(tert-butyldiphenylsilyloxy)-2methoxybromobenzene.

5   The above compound (2 mmol) is dissolved in THF (20 mL) under an inert atmosphere, and 1N n-BuLi in hexane (2 mL) is added. After 1 hour, the solution is cooled to -78° and a solution 1-[2-(4-fluorophenyl)ethyl-4-formylpiperidine, prepared as described in EP 531410, (2 mmol) is added. After 1 hour, the solution is added to water and extracted with CH_2Cl_2. The organic phase is washed, dried and evaporated, and the residue is

10   chromatographed to afford α-[3-(tert-butyldiphenylsilyloxy)-2-methoxyphenyl]-1-[2-(4-fluorophenyl)ethyl]-4-piperidinemethanol.

Using a procedure similar to that described in EP 531410, the above compound (2 mmol) is dissolved in DMF (20 mL) and S-(+)-α-methoxyphenylacetic acid (2 mmol), dicyclohexylcarbodiimide (2 mmol) and 4-dimethylaminopyridine (50 mg) are added. The

15   progress of the reaction is monitored by tlc. When it is complete, the solution is added to water and extracted with CH_2Cl_2. The organic phase is washed, dried and evaporated to afford a residue containing the diastereomeric esters. The individual diastereomers are separated by chromatography, so affording the diastereomer. This compound (1 mmol) is dissolved in MeOH (20 mL) and a solution of K_2CO_3 (4 mmol) in water (5 mL) is added.

20   The progress of the reaction is monitored by tlc. When it is complete, the solution is added to water and extracted with CH_2Cl_2. The organic phase is washed, dried and evaporated, and the residue is chromatographed to afford the desired product. The product (1 mmol) is dissolved in THF (10 mL) and 1M Bu_4NF in THF (20 mL) is added. The progress of the reaction is monitored by tlc. When it is complete, the solution is added to water and

25   extracted with CH_2Cl_2. The organic phase is washed, dried and evaporated, and the resulting mixture is separated into the individual diastereomers by chromatography, and the ester group is hydrolyzed to afford the desired phenol.

The phenol (1 mmol) and the bromo compound (1 mmol) are dissolved in DMF (15 mL) and K_2CO_3 (300 mg) and KI (50 mg) are added. The mixture is heated at 60° and the

30   progress of the reaction is monitored by tlc. When it is complete, the cooled solution is added to water and extracted with CH_2Cl_2. The extract is dried and evaporated, and the residue is chromatographed to afford the desired compound.

EXAMPLE A8

4-(2,3-Dimethoxybenzoyl)-1-(tert-butoxycarbonyl)piperidine, prepared as described in EP 531410, (5 mmol) is dissolved in MeOH (50 mL) and NaBH₄ (10 mmol) is added. The

5 progress of the reaction is monitored by tlc. When it is complete, the solution is added to dilute HCl and extracted with EtOAc. The organic phase is washed, dried and evaporated, and the residue is chromatographed to afford α-(2,3-dimethoxyphenyl)-1-tert-butoxycarbonyl-4-piperidinemethanol.

Using a procedure similar to that described in EP 531410, the above compound (2

10 mmol) is dissolved in DMF (20 mL) and S-(+)-α-methoxyphenylacetic acid (2 mmol), dicyclohexylcarbodiimide (2 mmol) and 4-dimethylaminopyridine (50 mg) are added. The progress of the reaction is monitored by tlc. When it is complete, the solution is added to water and extracted with CH₂Cl₂. The organic phase is washed, dried and evaporated to afford a residue containing the diastereomeric esters. The individual diastereomers are

15 separated by chromatography, so affording the desired diastereomer. This compound (1 mmol) is dissolved in MeOH (20 mL) and a solution of K₂CO₃ (4 mmol) in water (5 mL) is added. The progress of the reaction is monitored by tlc. When it is complete, the solution is added to water and extracted with CH₂Cl₂. The organic phase is washed, dried and evaporated, and the residue is chromatographed to afford (R)-α-(2,3-dimethoxyphenyl)-1-

20 tert-butoxycarbonyl-4-piperidinemethanol.

The above compound (1 mmol) is dissolved in CH_2Cl_2 (10 mL) and TFA (5 mL) is added. The progress of the reaction is monitored by tlc. When it is complete, the solvents are removed under vacuum and the residue is dissolved in CH_2Cl_2. The solution washed with dilute NaOH, then the organic phase is washed, dried and evaporated, and the residue

5 is chromatographed to afford (R)-α-(2,3-dimethoxyphenyl)-4-piperidinemethanol.

(R)-α-(2,3-dimethoxyphenyl)-4-piperidinemethanol (1 mmol) (prepared in Example A5) , (R)-α-(2,3-dimethoxyphenyl) 1-[2-(4-carboxyphenyl)ethyl]-4-piperidinemethanol (1 mmol) and dicyclohexylcarbodiimide (1 mmol) are dissolved in DMF (10 mL). The

10 progress of the reaction is monitored by tlc. When it is complete, the solution is added to water and extracted with CH_2Cl_2. The extract is dried and evaporated, and the residue is chromatographed to afford the desired compound.

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EXAMPLE A9
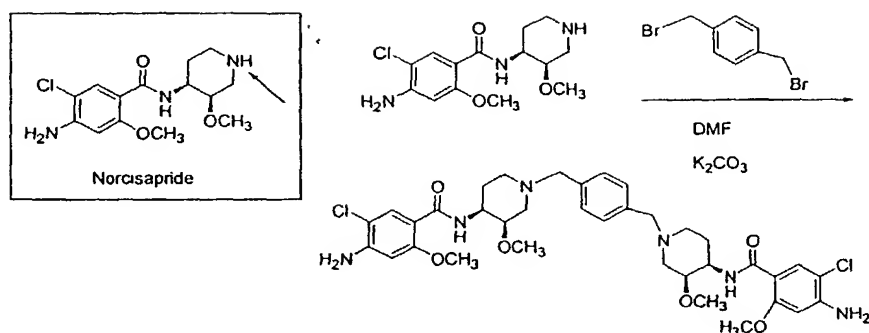
A mixture of 50 mmols of Norcisapride, described in WO 99/02496, 25 mmols of α,α'-dibromo-p-xylene and 50 mmols of potassium carbonate in 20 mL of DMF is warmed as necessary and the reaction followed by TLC. When judged complete, it is partitioned between isopropyl acetate and water and the aqueous phase back extracted with isopropyl acetate. The combined organic extracts are washed 3X water, dried over sodium sulfate, filtered and the solvent removed *in vacuo* to afford the desired compound, which may be purified by crystallization or chromatography.
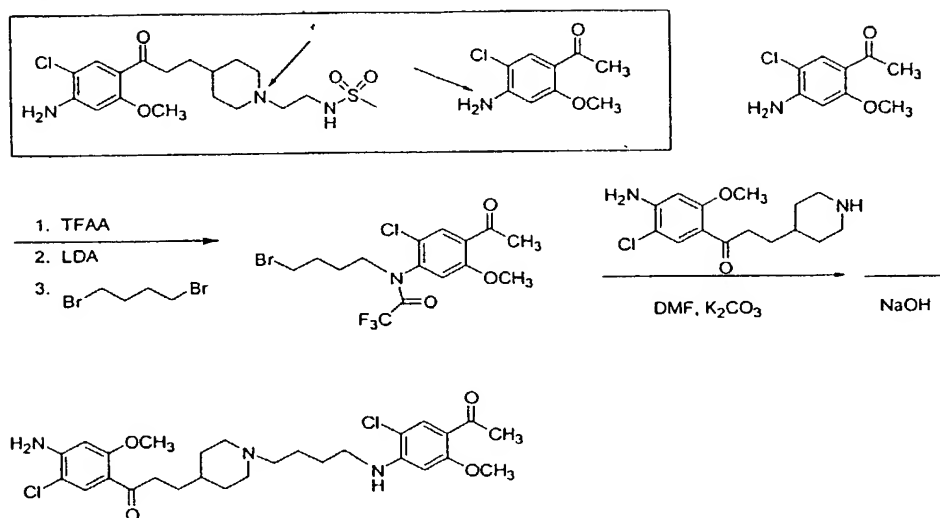
10

EXAMPLE A10

A solution of 50 mmols of the trifluoroacetamide of the compound described in Clark, R. *et al.* Bioorg. & Med. Chem. Lett., 1994, 4, 2477, in 100 mL of THF is cooled to -30C and 50 mL of 1 $\underline{N}$ LDA in THF added. After 30 min. 50 mmols of 1,4-dibromobutane is added and the reaction warmed to room temp. and heated further if necessary. When judged complete, it is concentrated and partitioned between isopropyl acetate and water and the aqueous phase back extracted with isopropyl acetate. The combined organic extracts are washed 3X water, dried over sodium sulfate, filtered and the solvent removed *in vacuo* to afford the desired compound after purification by crystallization or chromatography.

A mixture of 30 mmols of the above compound, 30 mmols of the piperidine described in Clark, R. *et al.* Bioorg. & Med. Chem. Lett., 1994, 4, 2477, and 30 mmols of potassium carbonate in 20 mL of DMF is warmed as necessary and the reaction followed by TLC. When judged complete, it is partitioned between isopropyl acetate and water and the aqueous phase back extracted with isopropyl acetate. The combined organic extracts are washed 3X water, dried over sodium sulfate, filtered and the solvent removed *in vacuo*. The residue is dissolved in 30 mL of methanol and 30 mL of 1 $\underline{N}$ NaOH added. When hydrolysis is complete, the reaction is concentrated *in vacuo* and the residue worked up with ethyl acetate and water. The crude product is purified by chromatography or crystallization to afford the desired compound.

EXAMPLE A11
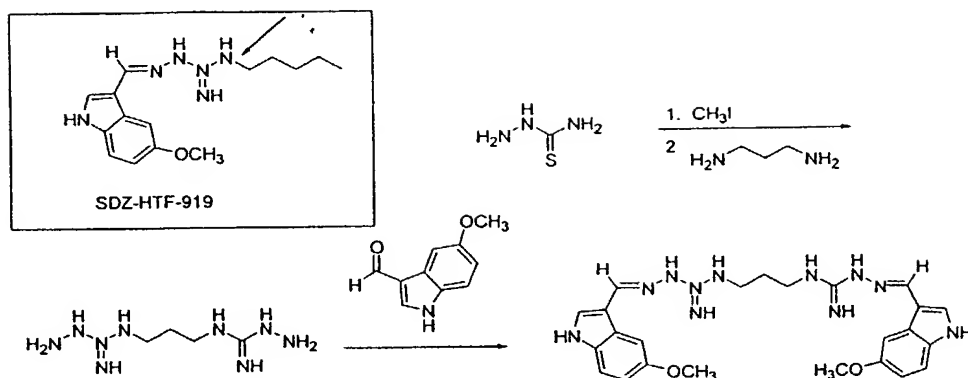
A mixture of 25 mmols of thiosemicarbazide in 100 mL of ethanol is treated with 30 mmols of iodomethane and 30 mmols of potassium carbonate. After 1hr., 12 mmols of 1,3-diaminopropane is added and the mixture heated to reflux. When complete, the reaction is cooled and the solution of the intermediate is used without further purification.

A solution of the 25 mmols of the compound above and 50 mmols of the aldehyde described in Buchheit, K. *et al.* J. Med. Chem., 1995, 38, 2331, in ethanol is acidified to pH 3-4 with conc. HCl and the reaction refluxed for 2 hr. The reaction is concentrated and the residue converted to its hydrochloride salt with methanol and ethereal HCl. This material is purified by crystallization to afford the desired compound.

EXAMPLE A12
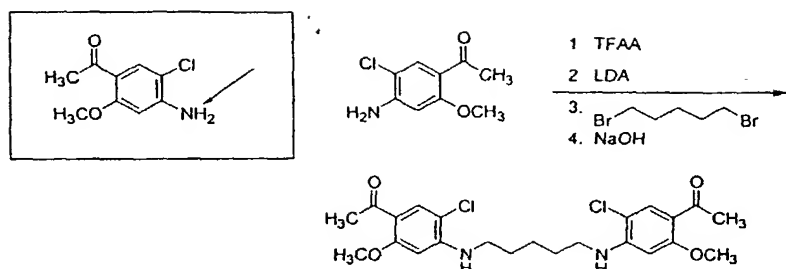
A solution of 30 mmols of the trifluoroacetamide of the compound described in Clark, R., *et al.* Bioorg. & Med. Chem. Lett., 1994, 4, 2477, in 50 mL of THF is cooled to -30C and an equivalent of LDA in THF is added. After 30 min. 15 mmols of 1,5-dibromopentane is added and the reaction warmed as necessary. When complete, an equivalent of 1 $\underline{N}$ NaOH is added and the mixture warmed to complete hydrolysis. The reaction is concentrated and worked up in the usual way with isopropyl acetate and sat. sodium bicarbonate to afford the desired compound upon purification by chromatography or crystallization.

EXAMPLE A13

SB-207266
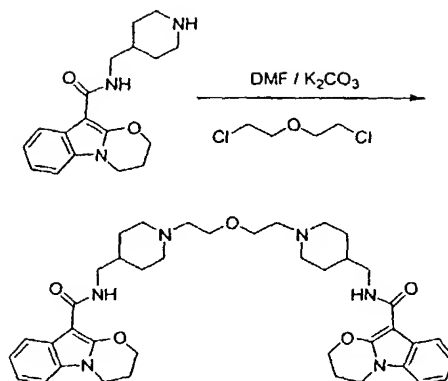
A mixture of 50 mmols of the starting compound, described in Gaster, L. *et al.* Bioorg. & Med. Chem. Lett., 1994, 4, 667 and in Gaster, *et al.* J. Med. Chem. 1995, 38, 4760, 25 mmols of bis-2-chloroethyl ether and 50 mmols of potassium carbonate in 20 mL of DMF is warmed as necessary and the reaction followed by TLC. When judged complete, it is partitioned between isopropyl acetate and water and the aqueous phase back extracted with isopropyl acetate. The combined organic extracts are washed 3X water, dried over sodium sulfate, filtered and the solvent removed *in vacuo* to afford the desired compound upon purification by crystallization or chromatography.

EXAMPLE A14
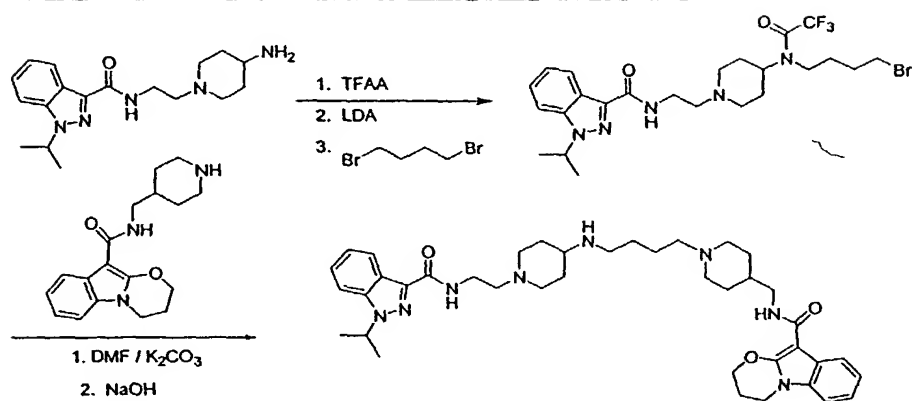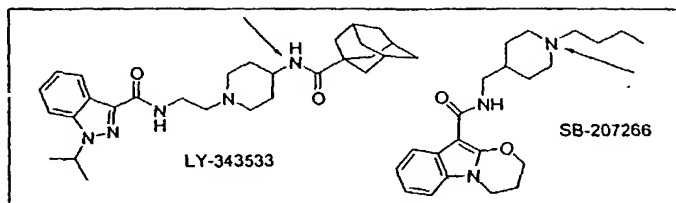
A solution of 50 mmols of the trifluoroacetamide of the starting compound,described in Schaus, J. *et al.* J Med. Chem., 1988, 41, 1943, in 100 mL of THF is cooled to –30 C and 50 mL of 1 $\underline{N}$ LDA in THF added. After 30 min. 50 mmols of 1,4-dibromobutane is added and the reaction warmed to room temp and heated further if necessary. When judged complete, it is concentrated and partitioned between isopropyl acetate and water and the aqueous phase back extracted with isopropyl acetate. The combined organic extracts are washed 3X water, dried over sodium sulfate, filtered and the solvent removed *in vacuo* to afford the intermediate, which may be purified by crystallization or chromatography.

A mixture of 30 mmols of the above intermediate , 30 mmols of the compound described in Gaster, L. *et al.* Bioorg. & Med. Chem. Lett., 1994, 4, 667 and in Gaster, *et al.* J. Med. Chem. 1995, 38, 4760, and 30 mmols of potassium carbonate in 20 mL of DMF is warmed as necessary and the reaction followed by TLC. When judged complete, it is partitioned between isopropyl acetate and water and the aqueous phase back extracted with isopropyl acetate. The combined organic extracts are washed 3X water, dried over sodium sulfate, filtered and the solvent removed *in vacuo*. The residue is dissolved in 30 mL of methanol and 30 mL of 1 $\underline{N}$ NaOH added. When hydrolysis is complete, the reaction is concentrated *in vacuo* and the residue worked up with ethyl acetate water. The crude product is purified by chromatography or crystallization to afford the desired material.

EXAMPLE A15

SB-207266
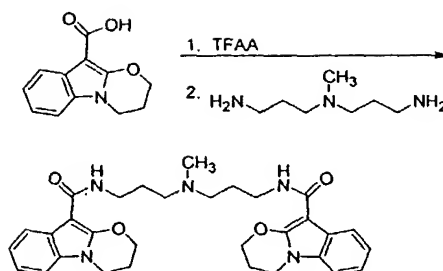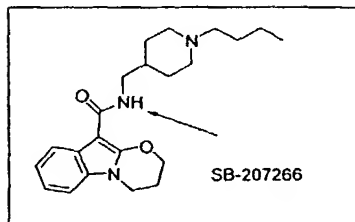
The acid, described in Gaster, L. *et al.* Bioorg. & Med. Chem. Lett., 1994, 4, 667 and in Gaster, *et al.* J. Med. Chem. 1995, 38, 4760, is prepared by simple hydrolysis of the compound and is converted to its trifluoroacetic anhydride in THF with TFAA and triethylamine in the usual manner. To the THF solution of the anhydride is added 0.5 equivalents of 3,3'-diamino-N-methyldipropylamine. The reaction is warmed as necessary and when complete is concentrated and the residue worked up in the usual manner with isopropyl acetate and water to afford the desired material which may be purified by chromatography or crystallization.

EXAMPLE A16
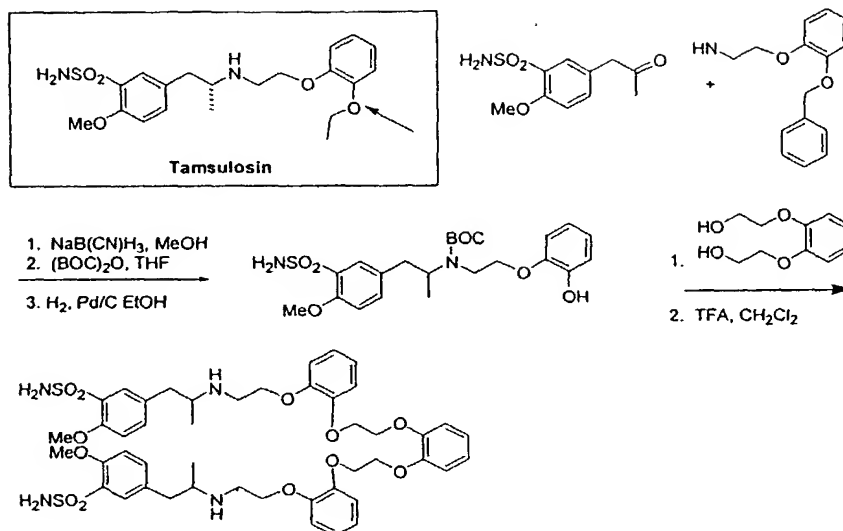
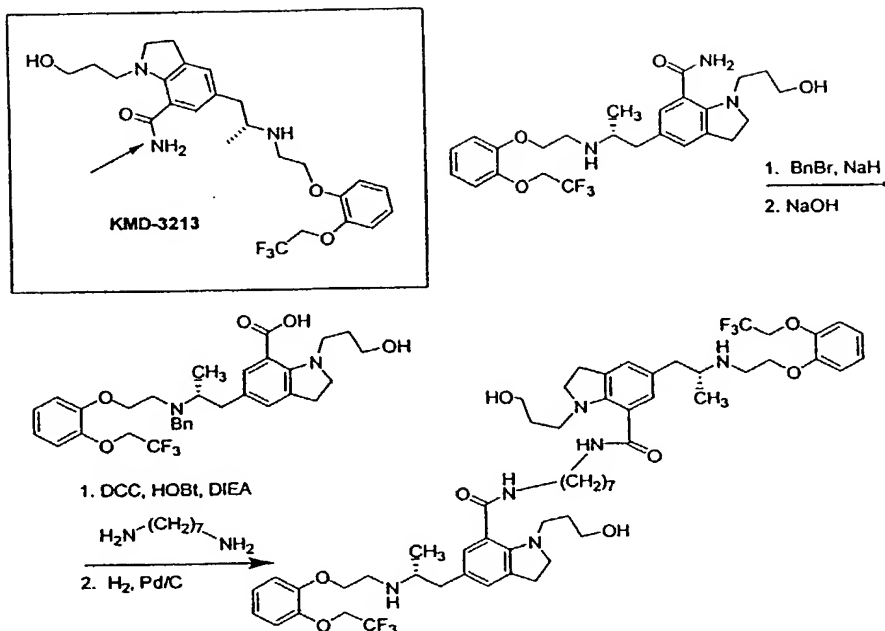A solution of 1-(2-aminoethoxy)-2-benzyloxybenzene (*Chem. Pharm. Bull.* 1988, *36*, 4121-35; 10 mmol) in methanol (40 mL) is acidified with acetic acid to pH 6.6 (pH meter) under a nitrogen atmosphere. 1-(4-Methyoxy-3-aminosulfonyl)phenyl-2-oxopropane (*Chem. Pharm. Bull.* 1992, *40*, 1443-51; 10 mmol) is added neat followed by sodium cyanoborohydride (11 mmol). The course of the reaction is followed by thin layer chromatography. After reaction occurs, the reaction solution is quenched in water and the pH of the aqueous mixture is adjusted to 9-10 with aqueous Na$_2$CO$_3$. The mixture is extracted with ether, the organic extracts are washed with half-saturated saline, dried (Na$_2$SO$_4$), filtered and concentrated under reduced pressure to give the crude product. The desired compound is obtained by purification of the crude product by use of HPLC.

In the second step, a solution of BOC-anhydride (5 mmol) and triethylamine (0.1 mL) in CH$_2$Cl$_2$ (5 mL) is stirred under an inert atmosphere. To this is added a solution the product of the preceding reaction (2 mmol) in CH$_2$Cl$_2$ (2 mL) and the resulting solution is stirred. The progress of the reaction is followed by TLC and when judged complete, the reaction is quenched by the addition of aqueous Na$_2$CO$_3$. The mixture is extracted with CH$_2$Cl$_2$, the organic extracts are washed with half-saturated saline, dried (Na$_2$SO$_4$), filtered and concentrated under reduced pressure to give the crude product. The desired N-BOC-product is obtained by purification of the crude product by use of HPLC.

In the third step, a solution of the compound from the preceding reaction in ethyl alcohol (10 mL) is hydrogenated at atmospheric pressure in the presence of 10% palladium-on-carbon (100 mg) until TLC evidence shows that reaction is complete. The mixture is filtered through Celite and the filter pad is washed thoroughly with ethanol. The

5 combined filtrates are concentrated under reduced pressure to give the crude product. The desired compound is obtained by purification of the crude product with the use of HPLC.

Diethyl azodicarboxylate (2 mmol) is added dropwise via a syringe to a stirred solution of triphenylphosphine (2 mmol) in THF (5 mL) at room temperature. To this is added a

10 solution of the product from the preceding reaction (2 mmol) and 2,2'-(1,2-phenylenedioxy)diethanol (1 mmol) in THF (3 mL). The resulting solution is stirred at RT and the progress of the reaction is followed by TLC. After reaction occurs, solvent is removed by evaporation under reduced pressure and the residue is purified by HPLC, giving the desired compound.

15 In the second step, a solution of the product from the preceding reaction and trifluoroacetic acid (3 mL) in CH_2Cl_2 (5 mL) is stirred at room temperature. The progress of the reaction is followed by TLC. After reaction occurs, more CH_2Cl_2 is added and the solution is washed with aqueous Na_2CO_3 and with H_2O. The organic layer is dried (Na_2SO_4), filtered and concentrated under reduced pressure to give the crude product. The

20 desired compound is obtained by purification of the crude product with the use of HPLC.

EXAMPLE A17

Sodium hydride (4 mmol) is added to a solution of (R)-KMD-3213 (E.P. 0 600 675 B1;

2 mmol) in dry DMF (3 mL) and is stirred under an inert atmosphere. To this is added

5   benzyl bromide (5 mmol) and the resulting solution is stirred. The course of the reaction is

followed by TLC and when complete, the reaction is quenched by the careful addition of

cold water and is diluted with additional half-saturated brine. The aqueous phase is

extracted with methylene chloride, the organic extract is washed with water and brine and

is dried (Na$_2$SO$_4$), filtered and concentrated under reduced pressure to give the crude

10   product. The desired compound is obtained by purification of the crude product with the

use of HPLC.

A solution of the compound prepared in the preceding step (2 mmol) in ethanol (2 mL)

and aqueous 1M NaOH (4 mL) is stirred under an inert atmosphere and is warmed until

reaction occurs. The progress of the reaction is followed by TLC. When reaction is

15   complete, the pH of the solution is adjusted to between 1 and 2 by the addition of 1 N HCl.

The solution is then lyophilized and the crude reaction product is dried and used directly in

the next step described below.

A solution of the product (2 mmol) from the preceding reaction, 1,7-diaminoheptane (1

mmol), and 1-hydroxybenzotriazole (2.5 mmol) in dry DMF (5 mL) is cooled in an ice-

water bath and stirred under an inert atmosphere. To the stirred solution is added 1-ethoxy-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (2.1 mmol). The course of the reaction is followed by TLC. The cooling bath is removed and after reaction occurs, the reaction mixture is partitioned between methylene chloride and saturated aqueous

5   NaHCO3. The organic layer is washed with water and brine, dried and concentrated under reduced pressure. The desired product is obtained by purification of the crude product by use of HPLC. The product is used in the following reaction.

A solution of the product of the preceding reaction in ethyl acetate (10 mL) is hydrogenated at atmospheric pressure in the presence of 10% palladium-on-carbon (100

10   mg) until TLC evidence shows that reaction is complete. The mixture is filtered through Celite and the filter pad is washed thoroughly with ethyl acetate. The combined filtrates are concentrated under reduced pressure to give the crude product. The desired compound is obtained by purification of the crude product with the use of HPLC.

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EXAMPLE A18

KMD-3213

1. Br^CH₂CH₂CH(OTBDMS)CH₂CH₂^Br
   DMF, 50C
   ───────────────────────────→
2. TFA

1,5-Dibromo-3-pentanol-*O*-TBDMS is prepared as follows: *tert*-Butyldimethylsilyl chloride (0.1 mol) is added to a solution of 1,5-dibromo-3-pentanol (0.05 mol) and

5   imidazole (0.05 mol) in dry pyridine (10 mL) and the resulting solution is stirred at RT. The progress of the reaction is followed by TLC. When reaction is complete, water (25 mL) is added to the solution which is then concentrated by evaporation under reduced pressure (>25 mm Hg, 30°C). The residue is dissolved in EtOAc and the solution is extracted with saturated aq. CuSO₄ to remove residual pyridine. The EtOAc solution is

10   washed with water, dried (Na₂SO₄), filtered and concentrated under reduced pressure to give the crude product. The pure product is obtained by purification of the crude product by flash chromatography over silica gel.

A solution of (*R*)-5-[2-[N-Boc-2-[2-(2,2,2-trifluoroethoxy)phenoxy]ethylamino]propyl]indoline-7-carboxamide (EP *0 600 675*; 2

15   mmol) and 1,5-dibromo-3-pentanol-*O*-TBDMS (1 mmol), prepared as above, and diisopropylethylamine (0.2 mL) in DMF (3 mL) is stirred and warmed under an inert atmosphere. The progress of the reaction is followed by TLC and when reaction is complete, the solution is poured into aqueous 5% NaHCO₃ and the aqueous mixture is extracted with methylene chloride. The organic extract solution is dried (Na₂SO₄), filtered

20   and concentrated under reduced pressure to give the crude product. The desired compound is obtained by purification of the crude product by use of HPLC.

A solution of the compound prepared by the preceding reaction and trifluoroacetic acid (3 mL) in CH_2Cl_2 (5 mL) is stirred at room temperature. The progress of the reaction is followed by TLC. After reaction occurs, more CH_2Cl_2 is added and the solution is washed with aqueous Na_2CO_3 and with H_2O. The organic layer is dried (Na_2SO_4), filtered and concentrated under reduced pressure to give the crude product. The desired compound is obtained by purification of the crude product with the use of HPLC.

EXAMPLE A19
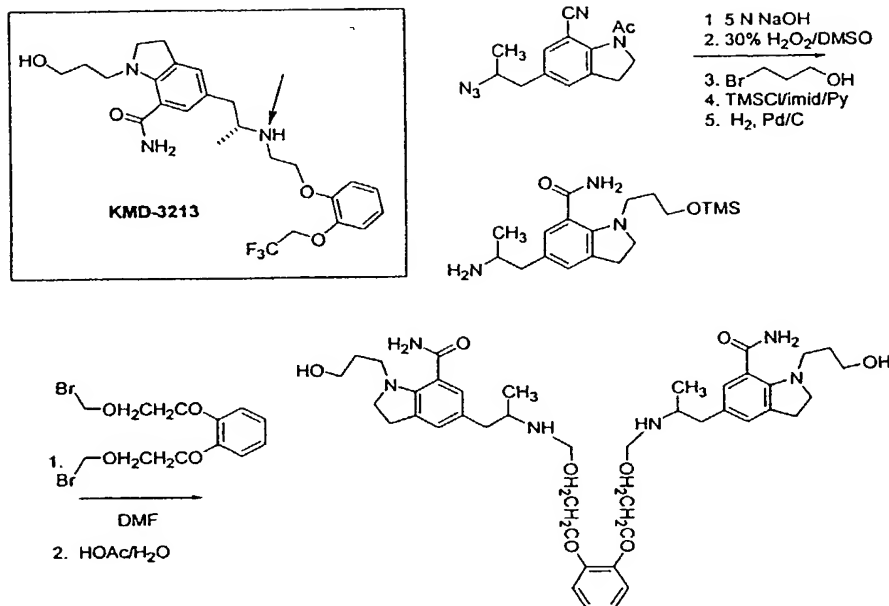
A solution of 1-acetyl-5-(2-azidopropyl)indoline-7-carbonitrile (*EP 0 600 675 B1;* 20 mmol) in aqueous 5 N NaOH (20 mL) and ethanol (40 mL) is stirred at room temperature. The course of the reaction is followed by TLC and when complete, the reaction solution is made alkaline by the addition of cold 1 N NaOH. The mixture is extracted with ether, the ether extracts are washed with water and with brine, dried (Na₂SO₄), filtered and concentrated under reduced pressure to give the crude product. The desired 5-(2-azidopropyl)indoline-7-carbonitrile is obtained by purification of the crude product with the use of HPLC.

To a solution of 5-(2-azidopropyl)indoline-7-carbonitrile (10 mmol) in DMSO (3 mL) is added 30% hydrogen peroxide (2.5 mL). The resulting mixture is stirred at room temperature for 20 minutes and then is transferred into a solution of aqueous 5 N NaOH (2.5 mL). This mixture is stirred at room temperature and the progress of the reaction is followed by TLC. When complete, the mixture is neutralized by the addition of acetic acid, water is added, and the mixture is extracted with ethyl acetate. The organic layer is washed with dilute aqueous sodium carbonate, with water and then is dried, filtered and concentrated under reduced pressure. The desired 5-(2-azidopropyl)indoline-7-carboxamide is obtained by purification of the crude product with the use of HPLC.

A solution of 5-(2-azidopropyl)indoline-7-carboxamide (8 mmol) together with 3-bromopropan-1-ol (8 mmol) in dioxane is heated and stirred with potassium carbonate. The progress of the reaction is followed by TLC and when complete, the solvent is removed by evaporation under reduced pressure. The residue is partitioned between dilute

5   aq. sodium bicarbonate and ethyl acetate. The organic extract layer is washed with water and with brine, is dried (Na_2SO_4), filtered and concentrated under reduced pressure to give the crude product. The desired 1-(3-hydroxypropyl)-5-(2-azidopropyl)indoline-7-carboxamide is obtained by purification of the crude product with the use of HPLC.

Trimethylsilyl chloride (4 mmol) is added to a solution of 1-(3-hydroxypropyl)-5-(2-

10   azidopropyl)indoline-7-carboxamide (2 mmol) and imidazole (2 mol) in dry pyridine (5 mL) and the resulting solution is stirred at RT. The progress of the reaction is followed by TLC. When reaction is complete, water (5 mL) is added to the solution which is then concentrated by evaporation under reduced pressure (>25 mm Hg, 30°C). The residue is dissolved in EtOAc and the solution is extracted with saturated aq. $CuSO_4$ to remove

15   residual pyridine. The EtOAc solution is washed with water, dried (Na_2SO_4), filtered and concentrated under reduced pressure to give the crude product. The pure 1-(3-hydroxypropyl)-5-(2-azidopropyl)indoline-7-carboxamide-O-TMS is obtained by purification of the crude product by flash chromatography over silica gel.

A solution of 1-(3-hydroxypropyl)-5-(2-azidopropyl)indoline-7-carboxamide-O-TMS

20   in ethyl acetate (10 mL) is hydrogenated at atmospheric pressure in the presence of 10% palladium-on-carbon (100 mg) until TLC evidence shows that reaction is complete. The mixture is filtered through Celite and the filter pad is washed thoroughly with ethyl acetate. The combined filtrates are concentrated under reduced pressure to give the crude product. The desired 1-(3-hydroxypropyl)-5-(2-aminopropyl)indoline-7-carboxamide-O-TMS is

25   obtained by purification of the crude product with the use of HPLC.

A solution 1-(3-hydroxypropyl)-5-(2-aminopropyl)indoline-7-carboxamide-O-TMS (2 mmol) and 1,2-bis(2-bromoethoxy)benzene (Aldrich; 1 mmol), and diisopropylethylamine (0.2 mL) in DMF (3 mL) is stirred and warmed under an inert atmosphere. The progress of the reaction is followed by TLC and when reaction is complete, the solution is poured into

30   aqueous 5% $NaHCO_3$ and the aqueous mixture is extracted with methylene chloride. The organic extract solution is dried (Na_2SO_4), filtered and concentrated under reduced pressure

to give the crude product. The desired compound is obtained by purification of the crude product by use of HPLC.
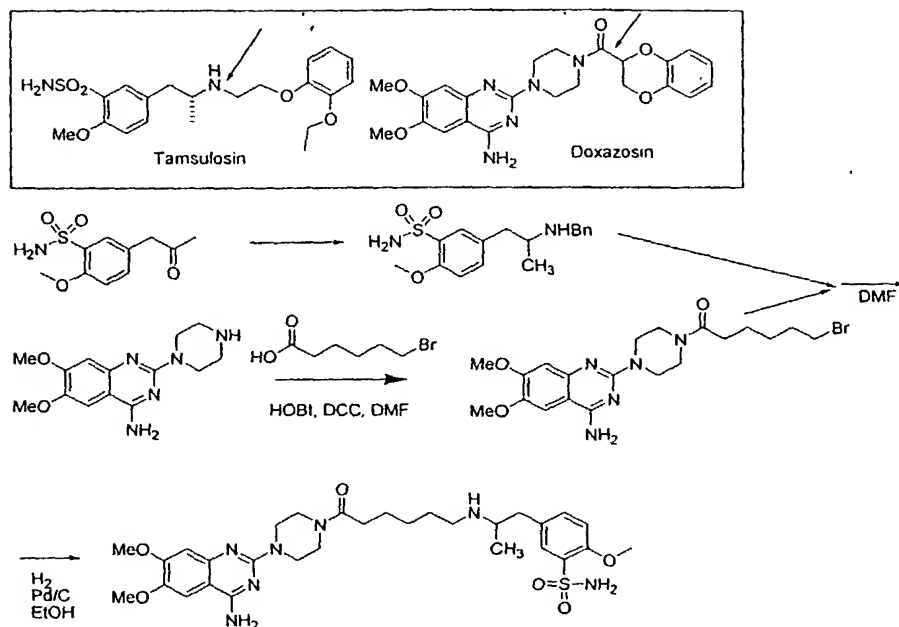
A solution of the product of the preceding reaction in HOAc-H_2O (4:1) (5 mL) is stirred under an inert atmosphere at room temperature. The reaction is followed by TLC and when complete, is diluted with EtOAc and washed several times with water and dilute aq. Na_2CO_3. The organic layer is dried (Na_2SO_4), filtered and concentrated under reduced pressure to give the crude product. The desired compound is obtained by purification of the crude product by use of HPLC.

EXAMPLE A20

A solution of 1-(3-hydroxypropyl)-5-(2-aminopropyl)indoline-7-carboxamide-*O*-TMS (2 mmol) (prepared in Example A19) in methanol (4 mL) is acidified with acetic acid to

5   pH 6.6 (pH meter) under a nitrogen atmosphere. Benzaldehyde (2.2 mmol) is added neat followed by sodium cyanoborohydride (2.1 mmol). The course of the reaction is followed by thin layer chromatography. After reaction occurs, the reaction solution is quenched in water and the pH of the aqueous mixture is adjusted to 9-10 with aqueous Na$_2$CO$_3$. The mixture is extracted with ether, the organic extracts are washed with half-saturated saline,

10   dried (Na$_2$SO$_4$), filtered and concentrated under reduced pressure to give the crude product. The desired compound is obtained by purification of the crude product by use of HPLC to be used in a later step.

A solution of 4-amino-1-butanol (2 mmol) in methanol (4 mL) is acidified with acetic

15   acid to pH 6.6 (pH meter) under a nitrogen atmosphere. 1-(4-Methyoxy-3-aminosulfonyl)phenyl-2-oxopropane (*Chem. Pharm. Bull. 1992, 40, 1443-51*; 2 mmol) is

added neat followed by sodium cyanoborohydride (2.1 mmol). The course of the reaction is followed by thin layer chromatography. After reaction occurs, the reaction solution is quenched in water and the pH of the aqueous mixture is adjusted to 9-10 with aqueous Na₂CO₃. The mixture is extracted with ether, the organic extracts are washed with half-

5   saturated saline, dried (Na₂SO₄), filtered and concentrated under reduced pressure to give the crude product. The desired compound is obtained by purification of the crude product by use of HPLC.

A solution of the product of the preceding reaction (2 mmol) in pyridine (5 mL) containing 4-dimethylaminopyridine (2-10 mg) is cooled in an ice bath and benzyl

10   chloroformate (0.5 mL) is added. The cooling bath is removed and the reaction solution is stirred at room temperature. Progress of the reaction is followed by tlc and when complete, the reaction is diluted with ethyl acetate, washed with 5% aq. sodium bisulfate, dried (Na₂SO₄), filtered and concentrated under reduced pressure to give the crude product. The desired compound is obtained by purification of the crude product by use of HPLC.
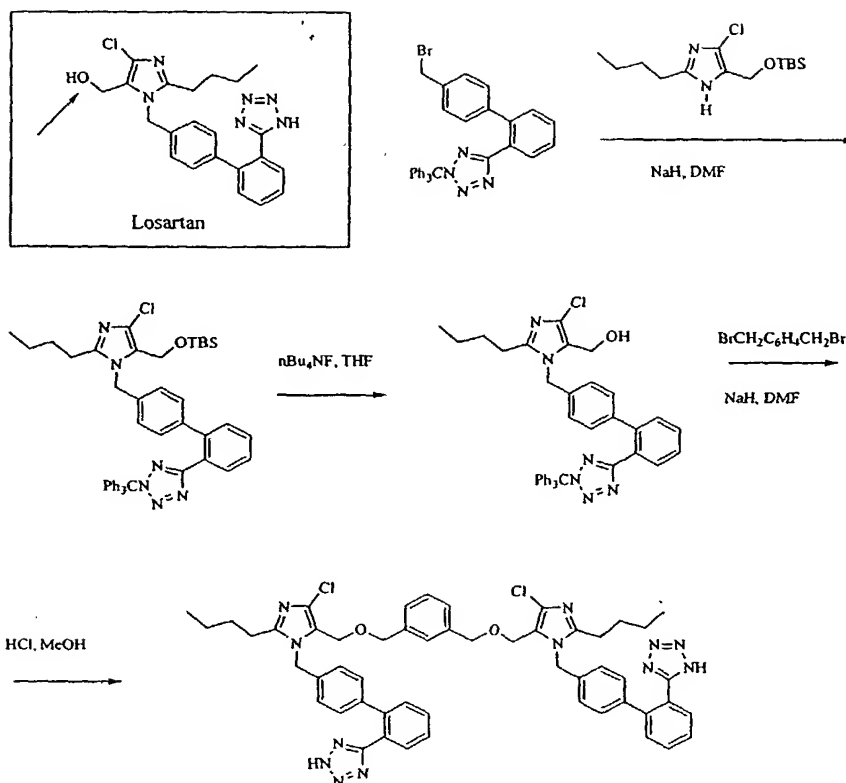
15   A solution of the product of the preceding reaction (2 mmol) and 4-methylmorpholine N-oxide (NMO, 3 mmol) in MeCN (10 mL) and methylene chloride (5 mL) is treated with 4-Å molecular sieves (50 mg) and stirred at ambient temperature for 10 min. Tetrapropylammonium perruthenate (TPAP, 0.05 mmol) is added and the reaction mixture is stirred at room temperature. The progress of the reaction is followed by tlc and when

20   complete, the reaction is diluted with methylene chloride and the mixture is filtered through silica gel. The filtrate is concentrated, giving the crude product.

A solution of the product the reaction described in the first paragraph, (2 mmol) in methanol (4 mL) is acidified with acetic acid to pH 6.6 (pH meter) under a nitrogen atmosphere. To this is added a solution of the aldehyde (2 mmol) prepared by the

25   preceding step in methanol (1 mL) followed by sodium cyanoborohydride (3 mmol). The course of the reaction is followed by thin layer chromatography. After reaction occurs, the reaction solution is quenched in water and the pH of the aqueous mixture is adjusted to 9-10 with aqueous Na₂CO₃. The mixture is extracted with ether, the organic extracts are washed with half-saturated saline, dried (Na₂SO₄), filtered and concentrated under reduced

30   pressure to give the crude product. The desired compound is obtained by purification of the crude product by use of HPLC.

A solution of the product of the preceding reaction in HOAc-H$_2$O (4:1) (5 mL) is
stirred under an inert atmosphere at room temperature. The reaction is followed by tlc and
when complete, is diluted with EtOAc and washed several times with water and dilute aq.
Na$_2$CO$_3$. The organic layer is dried (Na$_2$SO$_4$), filtered and concentrated under reduced

5   pressure to give the crude product. The desired compound is obtained by purification of
the crude product by use of HPLC.

A solution of the compound from the preceding reaction in ethyl acetate (10 mL) is
hydrogenated at atmospheric pressure in the presence of 10% palladium-on-carbon (100
mg) until tlc evidence shows that reaction is complete. The mixture is filtered through

10   Celite and the filter pad is washed thoroughly with ethyl acetate. The combined filtrates
are concentrated under reduced pressure to give the crude product. The desired compound
is obtained by purification of the crude product with the use of HPLC.

EXAMPLE A21

A solution of benzylamine (2 mmol) in methanol (4 mL) is acidified with acetic acid to pH 6.6 (pH meter) under a nitrogen atmosphere. 1-(4-Methoxy-3-aminosulfonyl)phenyl-2-oxopropane (*Chem. Pharm. Bull. 1992*, 40, *1443-51*; 2 mmol) is added neat followed by sodium cyanoborohydride (2.1 mmol). The course of the reaction is followed by thin layer chromatography. After reaction occurs, the reaction solution is quenched in water and the pH of the aqueous mixture is adjusted to 9-10 with aqueous Na$_2$CO$_3$. The mixture is extracted with ether, the organic extracts are washed with half-saturated saline, dried (Na$_2$SO$_4$), filtered and concentrated under reduced pressure to give the crude product. The desired compound is obtained by purification of the crude product by use of HPLC.

A solution of the compound prepared as described in Sekiya, Tetsuo, *et al. J. Med. Chem. 1983*, 26, 411-416, (1 mmol) and 1-(4-methyoxy-3-aminosulfonyl)phenyl-2-benzylaminopropane (1 mmol), prepared as described above, and diisopropylethylamine (0.2 mL) in DMF (3 mL) is stirred and warmed under an inert atmosphere. The progress of the reaction is followed by tlc and when reaction is complete, the solution is poured into aqueous 5% NaHCO$_3$ and the aqueous mixture is extracted with methylene chloride. The organic extract solution is dried (Na$_2$SO$_4$), filtered and concentrated under reduced pressure

to give the crude product. The desired compound is obtained by purification of the crude product by use of HPLC.

A solution of the compound of the preceding reaction in ethyl acetate (10 mL) is hydrogenated at atmospheric pressure in the presence of 10% palladium-on-carbon (100
5   mg) until tlc evidence shows that reaction is complete. The mixture is filtered through Celite and the filter pad is washed thoroughly with ethyl acetate. The combined filtrates are concentrated under reduced pressure to give the crude product. The desired compound is obtained by purification of the crude product with the use of HPLC.

EXAMPLE A22

Losartan

The synthesis of the TBS protected imidazole is cited in Greenlee, W. J., *Biorg. Med. Chem. Lett.*, 1993, 3(4), 557-660. The imidazole (604mgs, 2mmol) in DMF (10mls, *c.*

5 0.2$\underline{M}$) is treated with NaH (48mgs, 2mmol.) and the reaction is stirred at RT for 30

minutes. The biaryl bromide (300mgs, 1mmol.) is then added as a solution in DMF (5mls) and the reaction stirred for a further 60 minutes. The reaction is concentrated *in vacuo*. The crude reaction mixture is partitioned between ethyl acetate (25mls) and water (25mls). The organic layer is dried (MgSO$_4$), filtered and concentrated *in vacuo*. Flash chromatography

10 provides the desired material.

The silyl ether (1.02g, 2mmol.) is dissolved in THF (10mls, *c.* 0.2$\underline{M}$) and 1$\underline{M}$ TBAF in THF (3mls, 3mmol.) is added and the reaction is allowed to stir at room temperature for 2 hours. The reaction is concentrated *in vacuo*, and partitioned between ethyl acetate (25mls) and water (25mls). The organic layer is dried (MgSO$_4$), filtered and concentrated *in vacuo*.

15 The crude reaction mixture is purified by flash chromatography to provide the desired material.

The primary alcohol is dissolved in DMF (10mls, 0.2$\underline{M}$) and cooled to 0 C. NaH (48mgs, 2mmol.) is added and the reaction is stirred at this temperature for 30 minutes. α,α'-dibromo-m-xylene (260mgs, 1mmol.) is dissolved in DMF (10mls) and is added to the alkoxide solution *via* syringe pump over 60 minutes. The reaction is concentrated *in*

5   *vacuo*, and partitioned between ethyl acetate (25mls) and water (25mls). The organic layer is dried (MgSO$_4$), filtered and concentrated *in vacuo*. This crude reaction mixture is purified by flash chromatography to provide the desired multivalomer.

The dimeric protected tetrazole (1.17g, 1mmol.) is dissolved in methanol (5mls, *c.* 0.2$\underline{M}$) and is treated with 1$\underline{M}$ HCl in methanol (3mls, 3mmol.) and the reaction is stirred at

10   room temperature for 60 minutes. After this time, the reaction is concentrated *in vacuo*. The reaction is partitioned between ethyl acetate (25mls) and water (25mls). The organic layer is separated, dried (MgSO$_4$), filtered and concentrated *in vacuo*. The crude reaction mixture is purified by flash chromatography to provide the losartan multivalomer.

EXAMPLE A23

Losartan

NaOMe, MeOH, DMF

NaH, DMF

Bu₃SnN₃, xylene, reflux
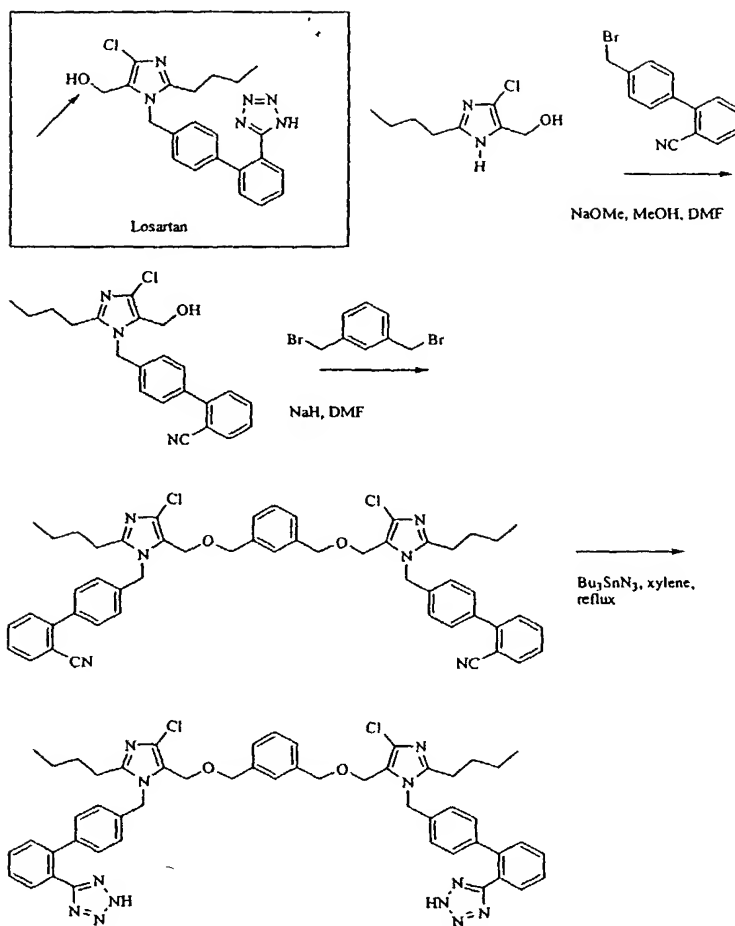
The imidazole (CAS Ref #79047-41-9) (276mgs, 2mmol) is added to a stirred solution of sodium methoxide (2mmol) in methanol (10mls) (2mmol, 46mgs of sodium dissolved in methanol) at 0C. The solvent is removed *in vacuo* and the so formed sodium salt of the imidazole is dissolved in DMF (10mls). The biaryl bromide (542mgs, 2mmol.) is added and the reaction is stirred at room temperature for 12 hours. The solvent is then removed *in vacuo*, and the reaction partitioned between ethyl acetate (25mls) and water (25mls). The organic layer is combined and the organic layer is dried with MgSO₄, the solvent in this removed *in vacuo*. Flash chromatography of the crude reaction mixture provides the alkylated imidazole.

Sodium hydride (48mgs, 2mmol.) is dissolved in DMF (10mls) and the alcohol (760mgs, 2mmol.) is added with stirring. This reaction is allowed to stir at room

temperature. The benzylic dibromide (261mgs, 1mmol.) in DMF is added dropwise *via* syringe pump over two hours. The reaction is allowed to stir at room temperature for a further two hours. The reaction is treated with aqueous NH$_4$Cl solution and partitioned between ethyl acetate (25mls) and water (25mls). The organic layer is separated, dried with

5   MgSO$_4$, filtered and concentrated *in vacuo*. This crude reaction mixture is purified by flash chromatography to provide the pure dimer.

The dimer (430mgs, 1mmol) is dissolved in xylene (20mls) and the tributylstannyl azide is added (615mg, 3mmol) and the reaction is heated to reflux in xylene (20mls) for 24 hrs. The solvent is removed *in vacuo* and the crude reaction mixture is treated with 2N

10   NaOH in methanol (20mls) to remove the N-stannyl group. The solvent is removed *in vacuo* and the reaction is dissolved in water and the solution neutralized (pH=7). The product is extracted with ethyl acetate (25mls x 3). The organic layer is dried with MgSO$_4$, filtered and concentrated *in vacuo*. The crude reaction mixture is purified by flash chromatography to provide the desired dimeric tetrazole.

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EXAMPLE A24
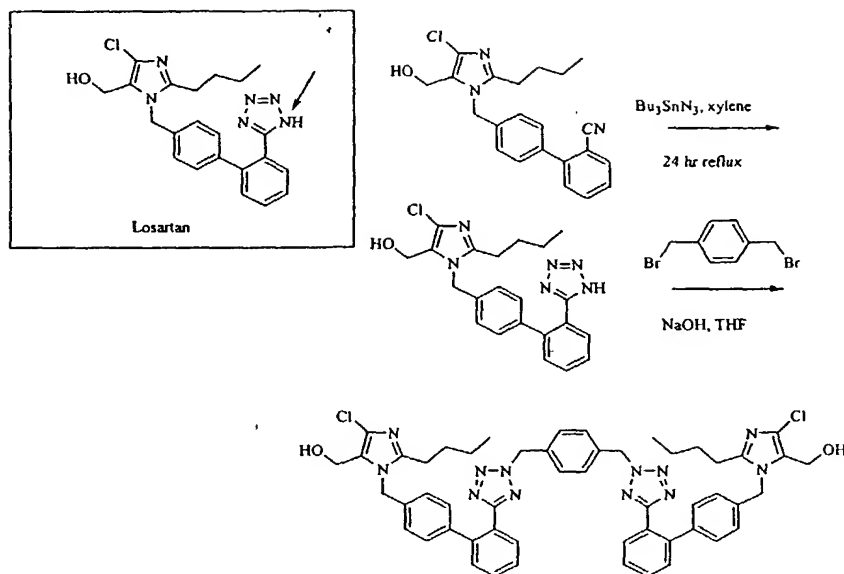
The biaryl nitrile, described in Example A23 (2mmol.) is dissolved in xylenes (10mls) and the tributylstannyl azide (4mmol) is added, the reaction is heated at reflux for 24 hrs, the reaction is allowed to cool and the solvent is removed *in vacuo*. The crude reaction mixture is treated with 1$\underline{N}$ NaOH in methanol (20mls) to hydrolyse the N-stannyl bond. The methanol is removed *in vacuo*, the crude reaction mixture is dissolved in water and neutralized with 1$\underline{M}$ HCl. The product is extracted from the aqueous phase with ethyl acetate (3 x 25mls). The organic layer is dried with $MgSO_4$, the drying agent is filtered, and the solvent is removed *in vacuo*. The crude reaction mixture can be purified by flash chromatography to provide the desired tetrazole.

The tetrazole (844mgs, 2mmol) is dissolved in DMF (5mls) and is treated with NaH (48mgs, 2mmol) and the reaction is stirred at RT for 20 minutes. α,α'-dibromo-p-xylene agent (260mgs, 1mmol.) in DMF (10mls) is added to tetrazole solution *via* syringe pump over one hour. The reaction is allowed to stir at room temperature for a further hour. The reaction is concentrated *in vacuo*, and is partitioned between ethyl acetate (25mls) and water (25mls). The organic layer is separated, dried ($MgSO_4$), filtered and concentrated *in vacuo*. The crude reaction mixture is purified by flash chromatography to provide the desired dimer.

EXAMPLE A25
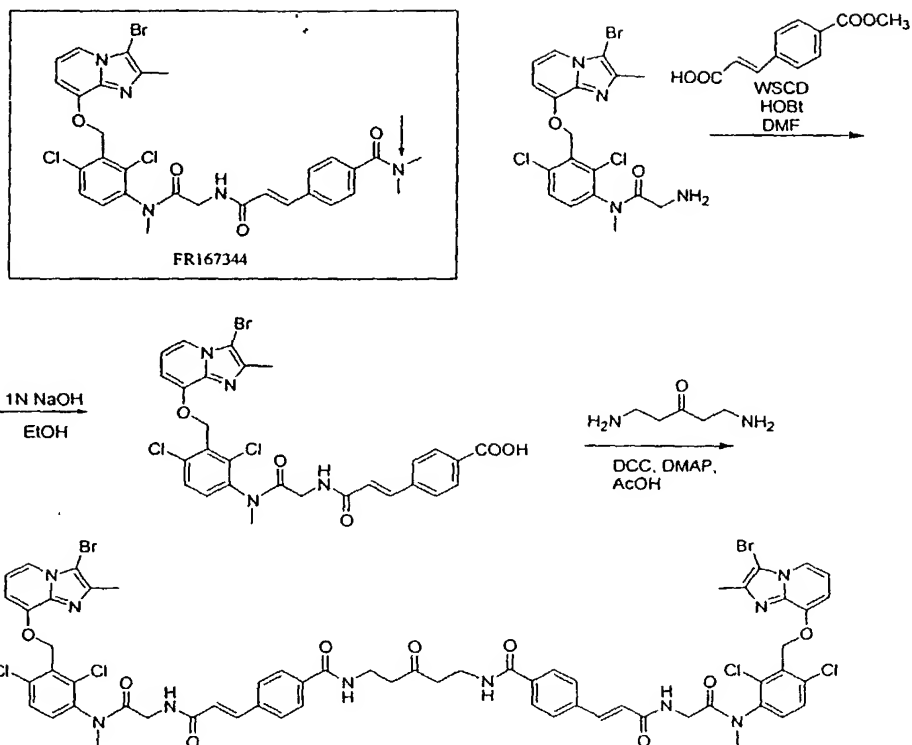
A solution of the carboxylic acid (2 mmols) (prepared in the first two steps by known technique) and 1,5-bisamino-3-oxapentane (1 mmol) in methylene chloride (20 mL) is

5   prepared under argon in a flask equipped with magnetic stirrer and drying tube. To this solution is added dicyclohexylcarbodiimide (solid, 2.1 mmols). The course of the reaction is followed by thin layer chromatography while stirring at room temperature. When reaction has occurred, the reaction solution is diluted with ethyl acetate and washed with water and with aqueous Na₂CO₃. The organic layer is dried (Na₂SO₄), filtered and

10   concentrated under reduced pressure to give the crude product. The desired compound is obtained by purification of the crude product by use of HPLC.

EXAMPLE A26

FR167344
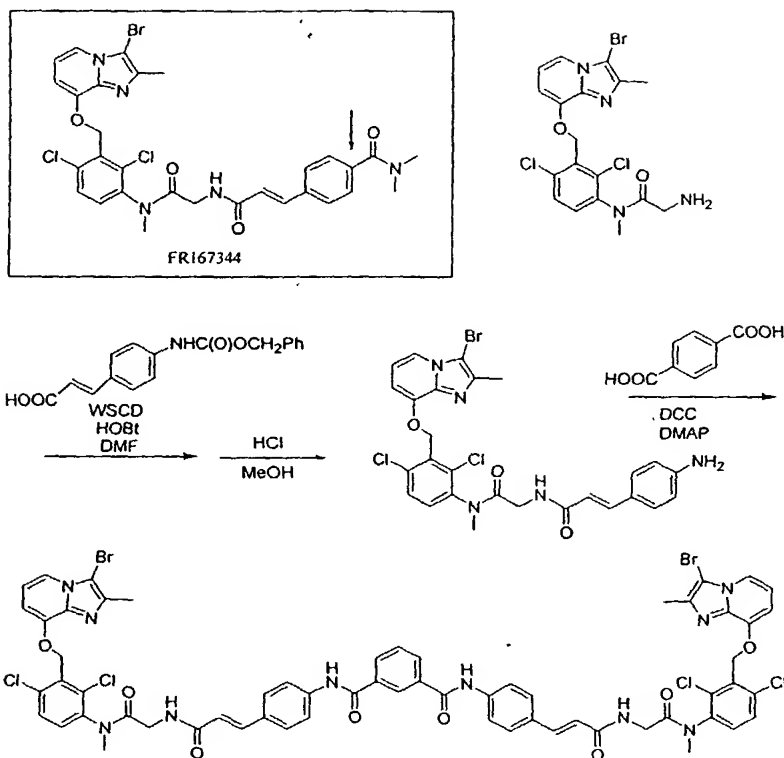
A solution of the compound prepared in the first two steps (2 mmols) and benzene-1,4-bisacetic acid (1mmol) in methylene chloride (20 mL) is prepared under argon in a flask

5   equipped with magnetic stirrer and drying tube. To this solution is added dicyclohexylcarbodiimide (solid, 2.1 mmols) while stirring at room temperature. The course of the reaction is followed by thin layer chromatography. When reaction has occurred, the reaction solution is diluted with ethyl acetate and washed with water and with aqueous Na_2CO_3. The organic layer is dried (Na_2SO_4), filtered and concentrated under

10   reduced pressure to give the crude product. The desired compound is obtained by purification of the crude product by use of HPLC.
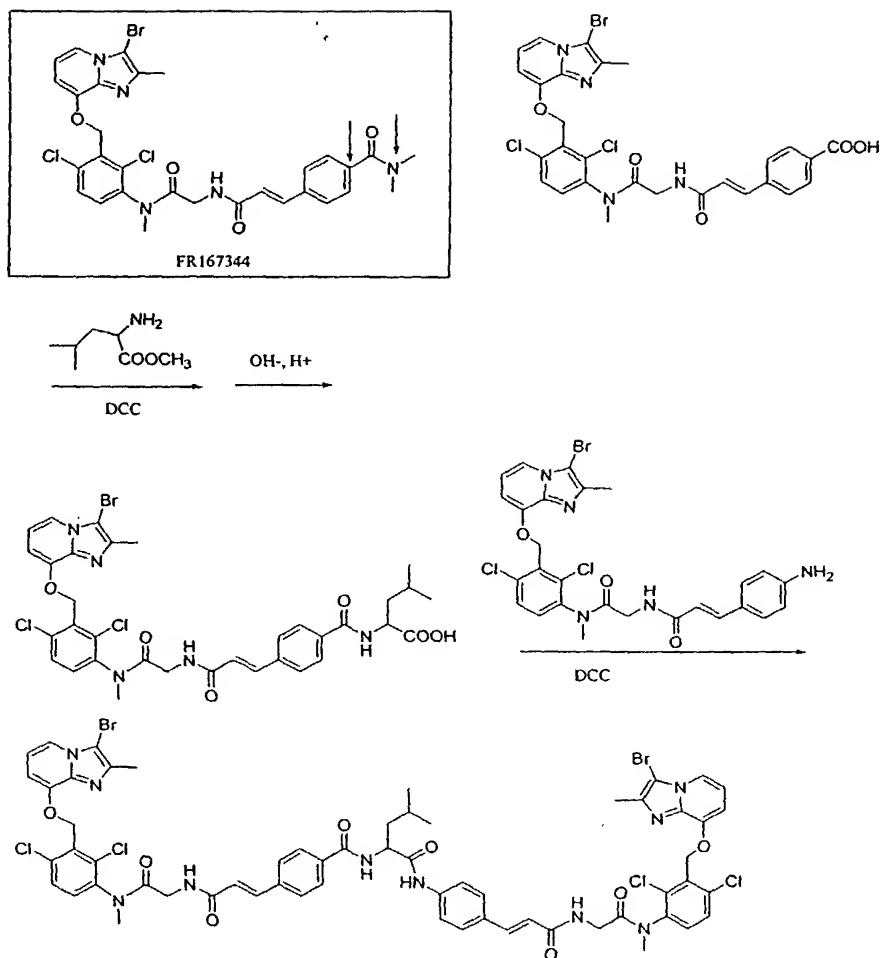
EXAMPLE A27

The starting material (described in Example A25) (1 mmol) and leucine methyl ester
(1mmol) in methylene chloride (20 mL) is prepared under argon in a flask equipped with

5 magnetic stirrer and drying tube. To this solution is added dicyclohexylcarbodiimide
(solid, 1.1 mmol). The course of the reaction is followed by thin layer chromatography
while stirring at room temperature. When reaction has occurred, the reaction solution is
diluted with ethyl acetate and washed with water and with aqueous Na₂CO₃. The organic
layer is dried (Na₂SO₄), filtered and concentrated under reduced pressure to give the crude

10 product. The desired compound is obtained by purification of the crude product by use of
HPLC.

A solution of the product of the preceding reaction and lithium hydroxide (100 mmols) in methanol (6 mL) and water (2mL) is stirred at room temperature. The reaction is followed by thin layer chromatography. When reaction has occurred, the pH of the solution is adjusted to 7 by the addition of dilute aq. hydrochloric acid. The solvent is
5   removed by lyophilization and the dry, crude product is used directly in the next reaction.

A solution containing the crude product from the preceding reaction and the compound prepared in Example A26 (1 mmol) in methylene chloride (20 mL) is prepared under argon in a flask equipped with magnetic stirrer and drying tube. To this solution is added dicyclohexylcarbodiimide (solid, 1.1 mmols). The course of the reaction is followed by
10   thin layer chromatography while stirring at room temperature. When reaction has occurred, the reaction solution is diluted with ethyl acetate and washed with water and with aqueous Na_2CO_3. The organic layer is dried (Na_2SO_4), filtered and concentrated under reduced pressure to give the crude product. The desired compound is obtained by purification of the crude product by use of HPLC.

EXAMPLE A28
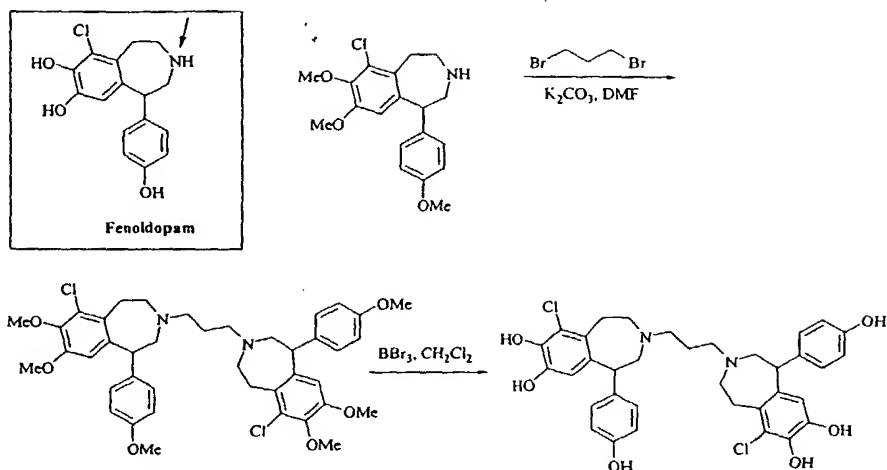
A solution of 20 mmols of 6-chloro-7,8-dimethoxy-1-(4-methoxyphenyl)-2,3,4,5-tetrahydro-1H-3-benzazepine (described in J. Med. Chem. 1982, 25, 352-358 and also in J.

5 Med. Chem. 1980, 23, 973-975) in DMF with 10 mmols of 1,3-dibromopropane and 20 mmols of potassium carbonate is heated as necessary and the reaction followed by TLC. When judged complete, the mixture is partitioned between ethyl acetate and water and the organic phase washed with water, dried over sodium sulfate and the solvent removed *in vacuo*. The residue is purified by chromatography to afford the desired product.

10 To a solution of the above product (4.6 mmol) in dry CH_2Cl_2 at $-10\ °C$, under argon, is added BBr_3 (12.8 mmol, 8 mL of a solution of 1 g of BBr_3 per 2.5 mL of CH_2Cl_2). The solution is allowed to come to 25 °C during the course of 30 min and the reaction is followed by TLC. The reaction is quenched by the addition of excess methanol, concentrated, and the residue is triturated with ethyl acetate, filtered, and dissolved in

15 warm water. The solution is adjusted to pH 8 with aqueous ammonia. Precipitated solid is filtered and washed with cold water. The solid is slurried in methanol and 98% methanesulfonic acid is added to give pH 1. Ethyl acetate is added to the resulting solution and then ether is added until the solution becomes cloudy. After cooling, crystallization affords the desired product.

20

-219-

EXAMPLE A29

SKF 75670

A suspension of 50% NaH (0.5 mmol) in mineral oil in 600 mL of Me$_2$SO is heated at

65-70 °C for 80 min. Then 300 mL of dry THF is added and the mixture is cooled in ice

5   water. A solution of trimethylsulfonium iodide (0.5 mmol) in 800 mL of Me$_2$SO is added

over 10 min. After the mixture is stirred for an additional 5 min, a solution of 3-

hydroxybenzaldehyde in 300 mL of THF is added over 10 min. The mixture is stirred at 0

°C for 15 min and then at 25 °C for 2 h, diluted with 8 L of ice water, and extracted several

times with ethyl acetate, and the extracts are washed with brine. The dried crude oxirane is

10   mixed with 2-(2-chloro-3,4-dimethoxyphenyl)ethylamine and heated at 110 °C for 18 h

under nitrogen. The cooled reaction mixture is triturated with ethyl acetate to give a

voluminous solid, which is collected and washed with ethyl acetate/petroleum ether.

Purification via column chromatography affords the desired product.

In 1000 mL of CF$_3$COOH is dissolved 0.4 mmol of the above product, and then 50 mL

15   of H$_2$SO$_4$ is added. The solution is refluxed for 2 h, concentrated *in vacuo*, basified with

cold NaOH solution and extracted with ethyl acetate. After the solution is washed with water, the dried, concentrated product is converted to the hydrochloride salt with ethereal HCl to afford the desired product as the hydrochloride salt.

A solution of 0.3 mmol of the above product, 0.53 mL of 37% HCHO, and 0.64 mL of

5   88% HCOOH is heated on the steam bath for 4.5 h, diluted with ice, and basified with NaOH solution, and the product is extracted into ethyl acetate and washed with water. The crude material is purified by chromatography to afford the desired product.

Resolution of the racemic mixture is achieved using the procedure described in Acta Phar. Suec. (1983) Issue Suppl. 2, Dopamine Receptor: Agonists 2, 132-150 to afford the

10   desired product.

A solution of 0.2 mmols of the above product in DMF with 0.1 mmols of 1,4-dibromobutane and 2.0 mmols of potassium carbonate is heated as necessary and the reaction followed by TLC. When judged complete, the mixture is partitioned between ethyl acetate and water and the organic phase washed with water, dried over sodium sulfate

15   and the solvent removed *in vacuo*. The residue is purified by chromatography to afford the desired product.

To a solution of the above product (0.1 mmol) in dry CH_2Cl_2 at −10 °C, under argon, is added BBr_3 (0.1 mmol, 0.07 mL of a solution of 1 g of BBr_3 per 2.5 mL of CH_2Cl_2). The solution is allowed to come to 25 °C during the course of 30 min and the reaction is

20   followed by TLC. The reaction is quenched by the addition of excess methanol, concentrated, and the residue is triturated with ethyl acetate, filtered, and dissolved in warm water. The solution is adjusted to pH 8 with aqueous ammonia. Precipitated solid is filtered and washed with cold water. The solid is slurried in methanol and 98% methanesulfonic acid is added to give pH 1. Ethyl acetate is added to the resulting solution

25   and then ether is added until the solution becomes cloudy. After cooling, crystallization affords the desired product.

EXAMPLE A30

A solution of 20 mmols of the starting material (prepared in Example A29) in DMF
with 20 mmols of 1,5-dibromopentane and 20 mmols of potassium carbonate is heated as

5   necessary and the reaction followed by TLC. When judged complete, the mixture is
partitioned between ethyl acetate and water and the organic phase washed with water, dried
over sodium sulfate and the solvent removed *in vacuo*. The residue is purified by
chromatography to afford the desired product.

A solution of 20 mmols of the above product in DMF with 20 mmols of the compound

10   described in Acta Phar. Suec. (1983) Issue Suppl. 2, Dopamine Receptor: Agonists 2, 132-
150and 20 mmols of potassium carbonate is heated as necessary and the reaction followed
by TLC. When judged complete, the mixture is partitioned between ethyl acetate and
water and the organic phase washed with water, dried over sodium sulfate and the solvent
removed *in vacuo*. The residue is purified by chromatography to afford the desired

15   product.

To a solution of the above product (4.6 mmol) in dry CH_2Cl_2 at –10 °C, under argon, is
added BBr_3 (12.8 mmol, 8 mL of a solution of 1 g of BBr_3 per 2.5 mL of CH_2Cl_2). The
solution is allowed to come to 25 °C during the course of 30 min and the reaction is
followed by TLC. The reaction is quenched by the addition of excess methanol,

concentrated, and the residue is triturated with ethyl acetate, filtered, and dissolved in warm water. The solution is adjusted to pH 8 with aqueous ammonia. Precipitated solid is filtered and washed with cold water. The solid is slurried in methanol and 98% methanesulfonic acid is added to give pH 1. Ethyl acetate is added to the resulting solution

5   and then ether is added until the solution becomes cloudy. After cooling, crystallization affords the desired dimeric product.
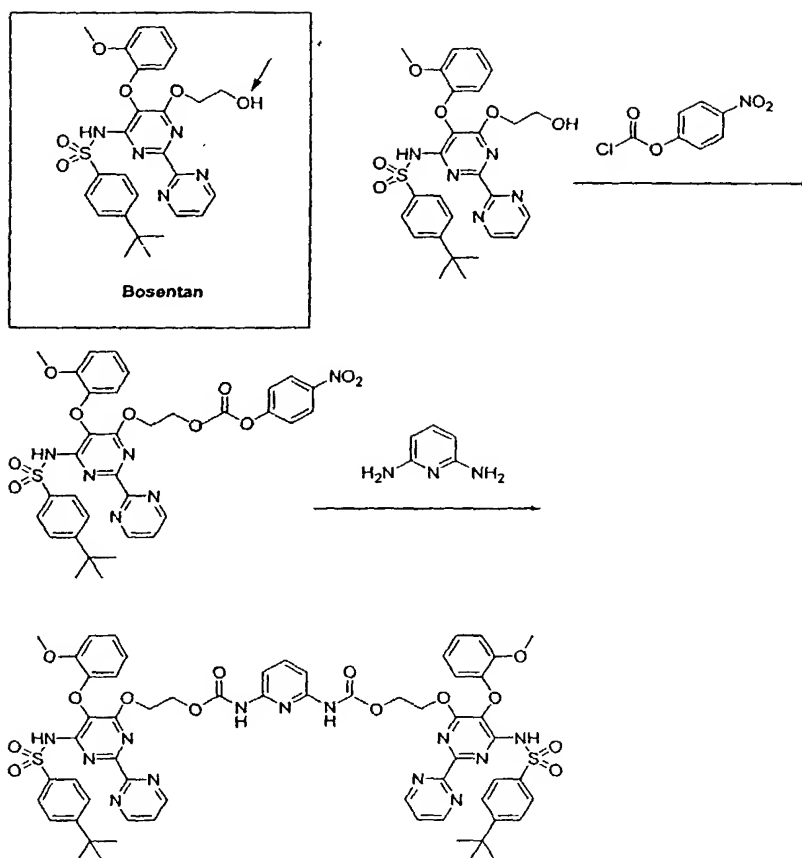
EXAMPLE-A31

A solution of 20 mmols of Bosentan in 50 mL of isopropyl acetate with 20 mmols of triethylamine at room temperature is treated with 20 mmols of 4-nitrophenyl
chloroformate. After 1hr., 10 mmols of 2,6-diaminopyridine is added and the reaction warmed and followed by TLC. When judged complete, water is added, the layers separated and the organic phase extracted sequentially with water, sat. sodium carbonate, and brine. After drying over sodium sulfate and filtering, the solvent is removed *in vacuo* to afford the desired compound, which is purified by chromatography.

EXAMPLE A32

SB 209670
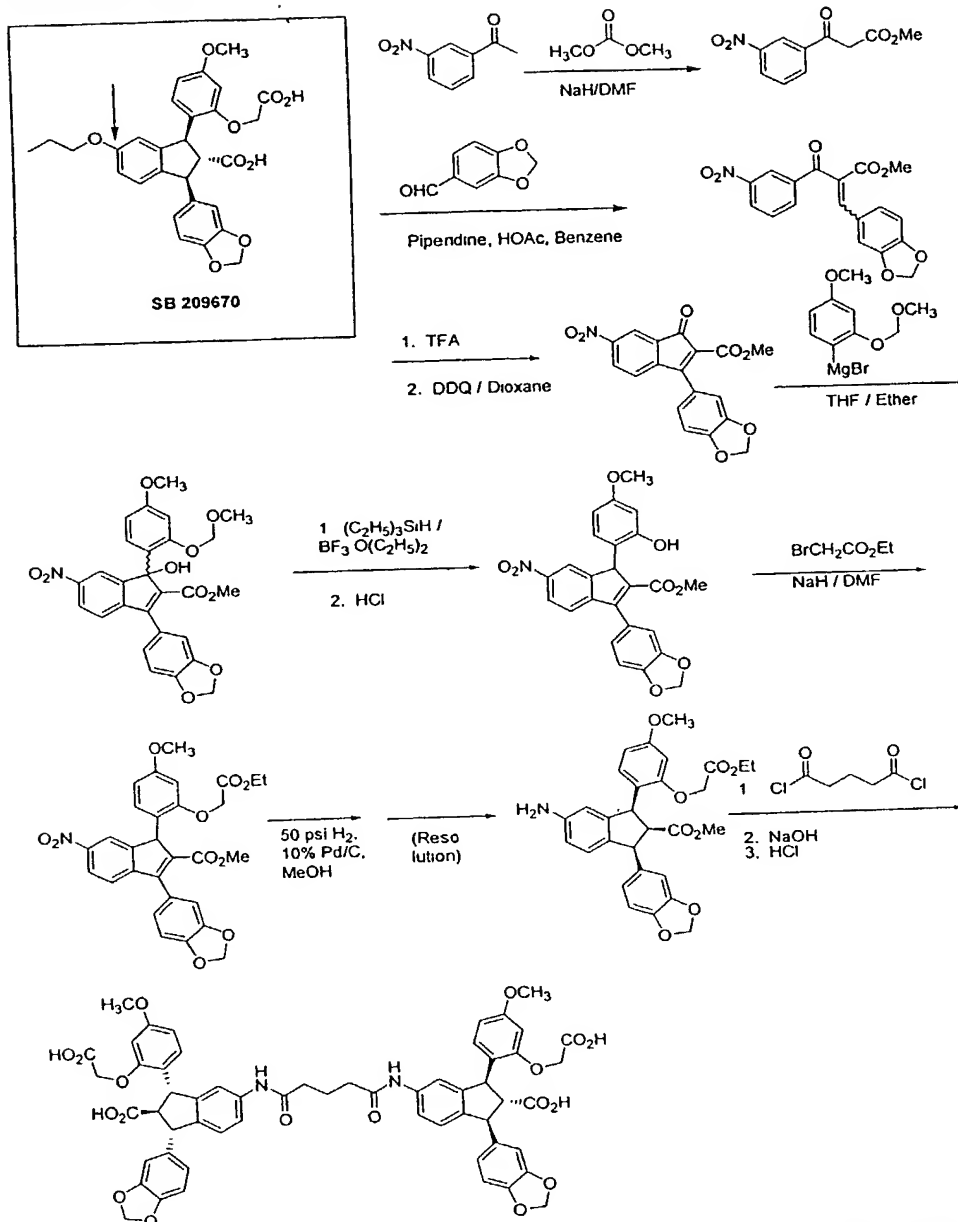
To a mixture of 5.0 mmols of oil free sodium hydride in 500 mL of dimethyl carbonate under N_2 is added over 30 min. a solution of 3'-nitroacetophenone in 800 mL of dimethyl carbonate. The mixture is then refluxed for 30 min., cooled and quenched by slow addition of 3 N HCl. The reaction is then partitioned between ethyl acetate and water and the

aqueous phase extracted with ethyl acetate. The combined organic phases are washed with water, aqueous sodium bicarbonate and dried over sodium sulfate. After filtering, the solvent is removed *in vacuo* to afford the desired product, which may be purified by crystallization or chromatography as necessary.

5   A mixture of 3.0 mmols of the above product, 3.4 mmols of piperonal, 41 mL of acetic acid and 14 mL of piperidine in 800 mL of benzene is refluxed with the azeotropic removal of water. After 4hrs., the mixture is concentrated *in vacuo* and the residue purified as necessary by crystallization or chromatography to afford the desired material.

To 1000 mL of trifluoroacetic acid at 0 °C under N_2 is added 19 mmols of the above

10   product. The mixture is warmed to room temperature and, after 30 min., concentrated under reduced pressure. The residue is partitioned between ethyl acetate and water and the organic phase washed successively with water, aqueous sodium bicarbonate and brine. After drying over sodium sulfate and filtering, the solvent is removed *in vacuo* and the residue purified as necessary by chromatography or crystallization. To a solution of 15

15   mmols of this material in 80 mL of dioxane cooled in an ice bath is added 15.5 mmols of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone. The mixture is stirred at room temperature for 2hrs. then for 1hr. at 50 C. The reaction is filtered and the solids washed with dioxane and the combined filtrates concentrated under reduced pressure. The residue is partitioned between ethyl acetate and water and washed successively with water, aqueous sodium

20   bicarbonate and brine. After drying over sodium sulfate and filtering, the solvent is removed *in vacuo* and the residue purified as necessary by crystallization or chromatography to afford the desired product.

A solution of 18 mmols of [4-methoxy-2-(methoxymethoxy)phenyl]magnesium bromide (J. Med. Chem., 1994, 37, 1553-1557) in ether is added to a solution of 12 mmols

25   of the above product in 80 mL of ether under N_2 at 0 C. The reaction is warmed to room temperature and after 10 min. partitioned between 1 N HCl and ethyl acetate and washed successively with water, aqueous sodium bicarbonate and brine. After drying and filtering, the solvent is removed *in vacuo* and the residue purified as necessary by chromatography or crystallization.

30   To a solution of 10 mmols of this material in 150 mL of methylene chloride at 0 °C under N_2 is added 13 mmols of triethylsilane followed by 49 mmols of boron trifluoride etherate. The resulting solution is stirred at 0 C for 10 min. and then partitioned between 1

N HCl and ethyl acetate. The organic phase is washed successively with water, aqueous sodium bicarbonate and brine. After drying over sodium sulfate and filtering, the solvent is removed *in vacuo* and the residue purified as necessary by chromatography or crystallization to afford the desired product.
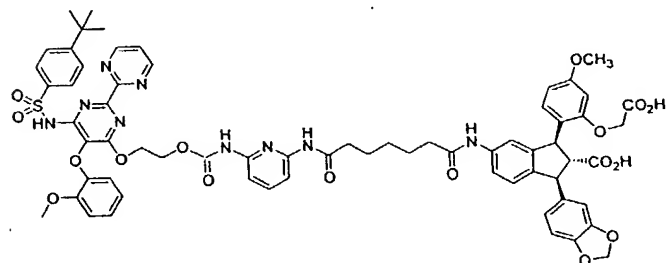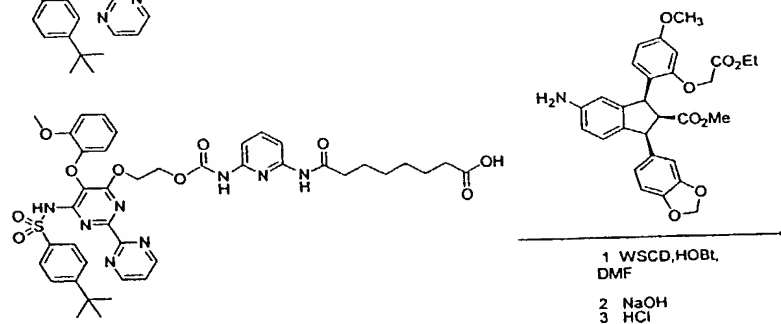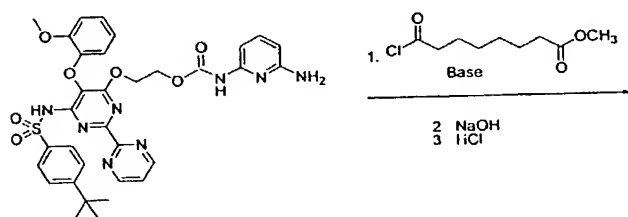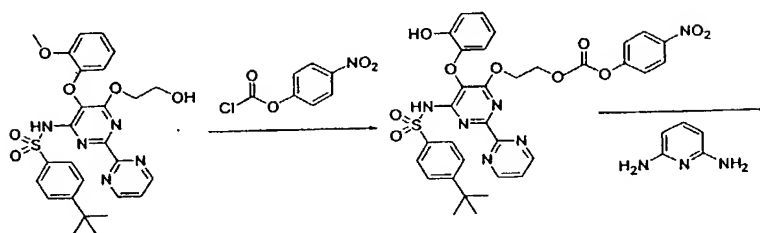
5   A solution of 5.0 mmols of the above product in 25 mL of DMF is added to a suspension of 6.0 mmols of oil free sodium hydride in 10 mL of DMF and the mixture stirred at room temperature for 10 min. The reaction is then treated with 6.0 mmols of ethyl bromoacetate and stirring continued for 20 min. followed by quenching with 3 N HCl and extraction with ethyl acetate. The organic phase is washed successively with water,

10   aqueous sodium bicarbonate and brine. After drying over sodium sulfate and filtering, the solvent is removed *in vacuo* and the residue purified as necessary by chromatography or crystallization to afford the desired product.

A solution of 2.5 mmols of the above product in 20 mL of methanol with 50 mg of 10% palladium on carbon is shaken under an atmosphere of 50 psi H_2 for 6hrs. After

15   exchanging for an atmosphere of N_2 and filtering, the solvent is removed *in vacuo* and the residue purified by crystallization or chromatography to afford the desired product.

Note: It is recognized that material produced by the above route will be racemic, but it is understood that a chiral product may be obtained by any of several methods, three of which are indicated here: 1. material may be separated by classical resolution by forming a

20   pair of diastereomeric salts with a chiral acid, such as dibenzoyltartaric acid, separating the diastereomers and freeing the individual enantiomers. 2. A preparative chiral HPLC column such as Chiralpak AD could be used to separate the enantiomers. 3. A chiral catalyst can be used in the hydrogenation to afford a single isomer directly.

A solution of 1.0 mmol of the product above in 20 mL of ethyl acetate with 1.0 mmol

25   of triethylamine is treated at room temperature with 0.5 mmols of glutaryl chloride at room temperature. After 1hr. the mixture is washed with water, dried over sodium sulfate, filtered and the solvent removed *in vacuo*. The resulting tetraester is purified as required and then dissolved in 25 mL of methanol and 15 mL of 2 N sodium hydroxide added. The reaction is warmed and followed by TLC. When judged complete, it is concentrated and

30   30 mL of 1N HCl is added and the mixture extracted with ethyl acetate which is washed with water, dried over sodium sulfate, filtered and the solvent removed *in vacuo*. The residue is purified by crystallization or chromatography to afford the desired product.

EXAMPLE A33

Bosentan

SB 209670

A solution of 20 mmols of Bosentan in 50 mL of isopropyl acetate with 20 mmols of triethylamine at room temperature is treated with 20 mmols of 4-nitrophenyl chloroformate. After 1hr., 10 mmols of 2,6-diaminopyridine is added and the reaction warmed and followed by TLC. When judged complete, water is added, the layers

5   separated and the organic phase extracted sequentially with water, sat. sodium carbonate, and brine. After drying over sodium sulfate and filtering, the solvent is removed *in vacuo* to afford the desired Bosentan derivative.
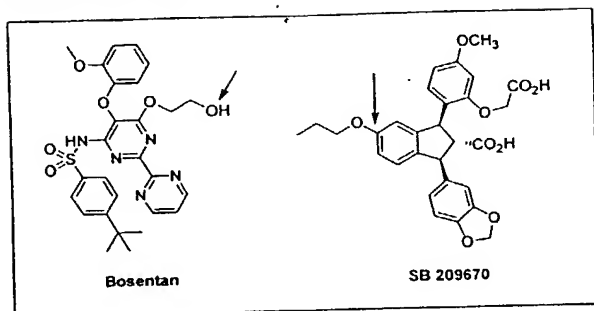
A solution of 10 mmols of the Bosentan derivative in 20 mL of ethyl acetate with 10 mmols of triethylamine is treated at room temperature with 10 mmols of methyl suberyl

10   chloride at room temperature. After 1 hr. the mixture is washed with water, dried over sodium sulfate, filtered and the solvent removed *in vacuo*. The resulting ester is purified as required and then dissolved in 25 mL of methanol and 10 mL of 2 $\underline{N}$ sodium hydroxide added. The reaction is warmed and followed by TLC. When judged complete, it is concentrated and 20 mL of 1$\underline{N}$ HCl is added and the mixture extracted with ethyl acetate

15   which is washed with water, dried over sodium sulfate, filtered and the solvent removed.

The product from the preceding reaction is carefully dried and dissolved in 20 mL of dry DMF and 10 mmols of the SB209670 derivative prepared in Example A32 and 14 mmols of 1-hydroxybenzotriazole added under N_2. The mixture is cooled in an ice bath and 11 mmols of 1-ethoxy-3-[3-(dimethylamino)propyl]carbodimiide hydrochloride added.

20   The cooling bath is removed and the reaction followed by TLC. When judged complete, the reaction mixture is partitioned between water and isopropyl acetate and the organic phase exhaustively washed with water and the solvent removed *in vacuo*. The resulting ester is purified as required and then dissolved in 25 mL of methanol and 15 mL of 2 $\underline{N}$ sodium hydroxide added. The reaction is warmed and followed by TLC. When judged

25   complete, it is concentrated and 30 mL of 1$\underline{N}$ HCl is added and the mixture extracted with ethyl acetate which is washed with water, dried over sodium sulfate, filtered and the solvent removed *in vacuo*. The residue is purified by crystallization or chromatography to afford the desired dimeric compound.

EXAMPLE A34

The starting compound is described in Miller, J., et. al. J. Org. Chem., 50, 2121-2123 (1985). 2-Chloroethanol (10 mmol) is added in portions to a solution of the above

5 compound (10 mmol) and triphenylphosphine (10 mmol) in anhydrous THF at 0 °C. The mixture is cooled to –20 °C and diethyl azodicarboxylate (DEAD) (12 mmol) is added dropwise over 30 min. During this time, the temperature of the mixture is not allowed to rise above –10 °C. When the addition is complete, the mixture is allowed to warm to room temperature and stirred for 16 h. The mixture is concentrated *in vacuo* and the resulting

10 residue is purified by chromatography to afford the desired product.

A solution of the product from the preceding step (1.9 mmols), N,N',N",-trimethylbis(hexamethylene)triamine (1 mmol) (CAS 86018-07-7), and diisopropylethylamine (DIPEA) (2.5 mmols) in DMF (10 mL) is maintained at reflux, and the reaction is monitored by TLC. When it is complete, the solution is added to water and

15 extracted with CH_2Cl_2. The extract is dried and evaporated, and the residue is chromatographed to afford the desired compound.

EXAMPLE A35
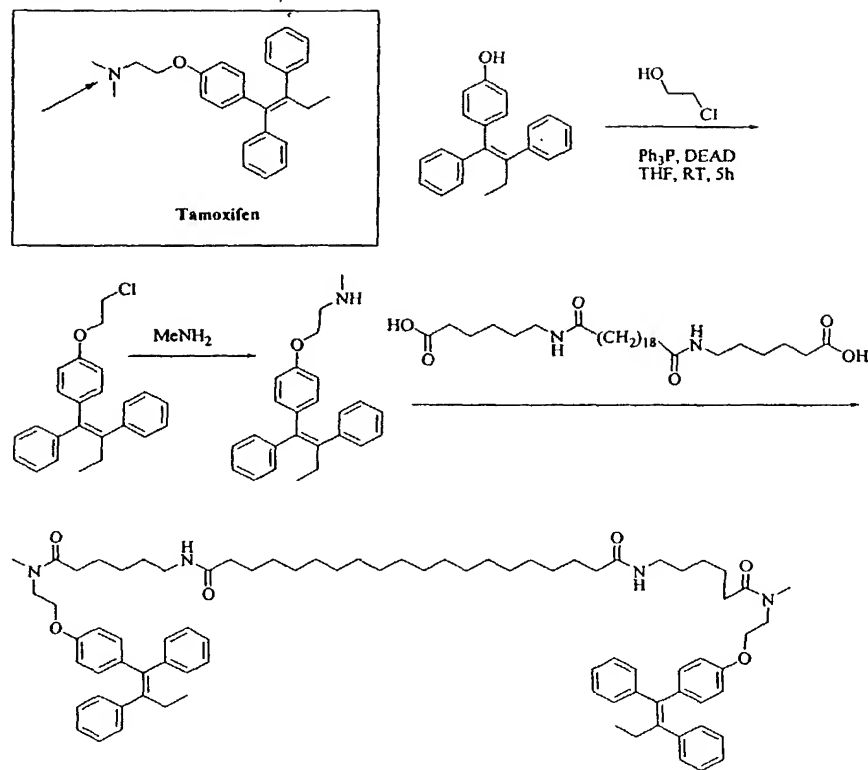
The starting compound is described in Miller, J., et. al. J. Org. Chem., 50, 2121-2123 (1985). 2-Chloroethanol (10 mmol) is added in portions to a solution of the above

5   compound (10 mmol) and triphenylphosphine (10 mmol) in anhydrous THF at 0 °C. The mixture is cooled to -20 °C and diethyl azodicarboxylate (DEAD) (12 mmol) is added dropwise over 30 min. During this time, the temperature of the mixture is not allowed to rise above -10 °C. When the addition is complete, the mixture is allowed to warm to room temperature and stirred for 16 h. The mixture is concentrated *in vacuo* and the resulting

10   residue is purified by chromatography to afford the desired product.

The above compound (5 mmol) is added to a solution of methylamine (2 g) in MeOH (40 mL). The progress of the reaction is monitored by TLC. When judged complete, the mixture is added to water and extracted with CH_2Cl_2. The extract is dried and evaporated and the residue is chromatographed to afford the desired product.

A solution of the above product (2 mmol) and the diacid (1 mmol) in methylene chloride (20 mL) is prepared under argon in a flask equipped with magnetic stirrer and drying tube. To this solution is added dicyclohexylcarbodiimide (solid, 2.1 mmols) while stirring at room temperature. The course of the reaction is followed by thin layer

5   chromatography. When reaction has occurred, the reaction solution is diluted with ethyl acetate and washed with water and with aqueous Na_2CO_3. The organic layer is dried (Na_2SO_4), filtered and concentrated under reduced pressure to give the crude product. The desired compound is obtained by purification of the crude product by use of HPLC.

EXAMPLE A36

Cetirizine
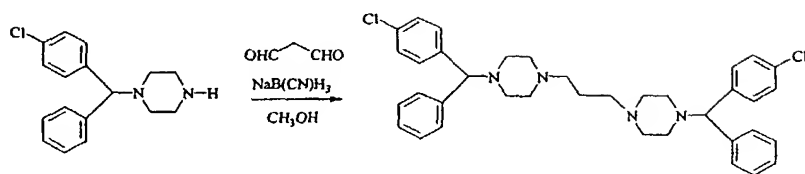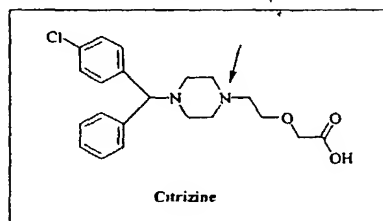
A solution of 1-(4-chlorobenzhydryl)piperazine (CAS 303-26-4; 2 mmols) in methanol (8 mL) is acidified with acetic acid to pH 6.5 (pH meter) under a nitrogen atmosphere.

5 Malonaldehyde (1 mmol) is added neat followed by sodium cyanoborohydride (3.1 mmols). The course of the reaction is followed by thin layer chromatography. After reaction occurs, the reaction solution is quenched in water and the pH of the aqueous mixture is adjusted to greater than 10 with aqueous NaOH. The mixture is extracted with ether, the organic extracts are washed with half-saturated saline, dried (Na$_2$SO$_4$), filtered

10 and concentrated under reduced pressure to give the crude product. The desired compound is obtained by purification of the crude product with the use of HPLC.

EXAMPLE A37
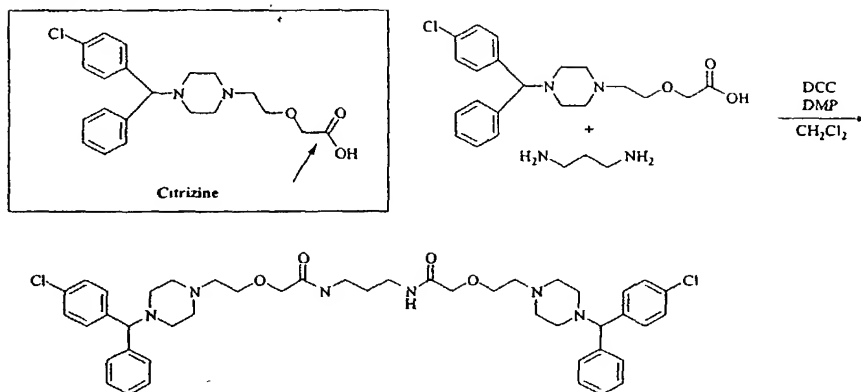
A solution of [2-[4-[(4-chlorophenyl)phenylmethyl]-1-piperazinyl]ethoxy]acetic acid piperazine (Opalka, C.J.; et.al., Synthesis 1995, 766-8; 2 mmol), 1,3-diaminopropane (1

5 mmol), and DMAP (10 mg) in CH₂Cl₂ (5 mL) is prepared under argon in a flask equipped with magnetic stirrer and a drying tube. To this solution is added dicyclohexylcarbodiimide (solid, 2.2 mmol). The progress of the reaction is followed by tlc and after reaction occurs, the reaction solution is quenched in water, aqueous sodium bicarbonate is added and the aqueous mixture is extracted with methylene chloride. The

10 organic layer is washed with aqueous Na_2CO_3 and with H_2O, dried (Na_2SO_4), filtered and concentrated under reduced pressure to give the crude product. The desired compound is obtained by purification of the crude product with the use of HPLC.

EXAMPLE A38

Norastemizole

Citrizine
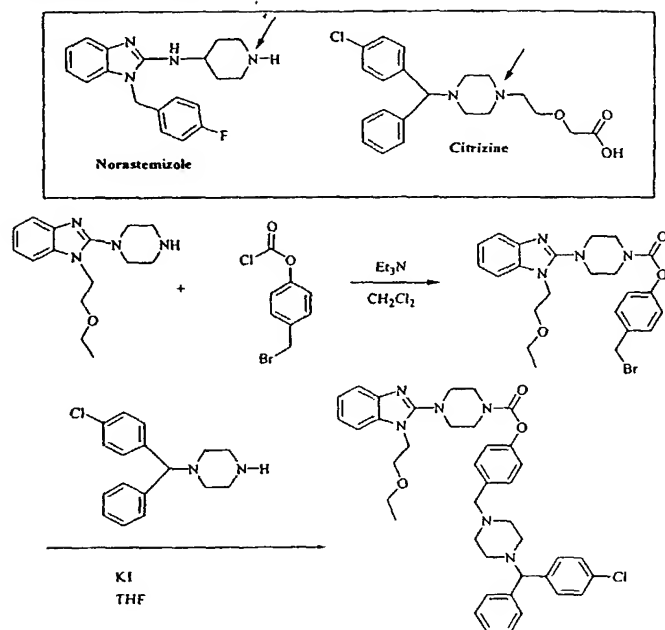
A solution of 4-(bromomethyl)benzylchloroformate (2 mmols) in CH₂Cl₂ (5 mL) containing Et₃N (0.2 mL) is stirred and cooled in an ice-water bath under an inert

5 atmosphere. To this is added dropwise a solution of 1-(2-ethoxyethyl)-2-(hexahydro-1*H*-1,4-diazepin-1-yl)-1*H*-benzimidazole (CAS 87233-69-0; 2 mmols) in CH₂Cl₂ (5 mL). After addition is complete, the cooling bath is removed and the reaction solution is allowed to warm to room temperature. The progress of the reaction is followed by tlc and when reaction has occurred, the reaction solution is quenched in cold 5% aqueous Na₂CO₃. The

10 layers are separated and the organic layer is washed with aqueous Na₂CO₃, with water and is dried (Na₂SO₄), filtered and concentrated under reduced pressure to give the crude product. The desired compound is obtained by purification with the use of HPLC.

A mixture of the compound prepared in the preceding reaction (2 mmols), 1-(4-chlorobenzhydryl)piperazine (CAS 303-26-4; 2 mmols), and KI (2 mmols) in THF (20

15 mL) is stirred under an inert atmosphere at RT. The progress of the reaction is monitored by TLC and when the reaction is complete, solvent is removed in vacuo. Water is mixed with the residue and is extracted with CH₂Cl₂. The organic extract is washed with half saturated brine, dried (Na₂SO₄), filtered and concentrated under reduced pressure giving the crude product. The desired compound is obtained by purification by HPLC.

EXAMPLE A39
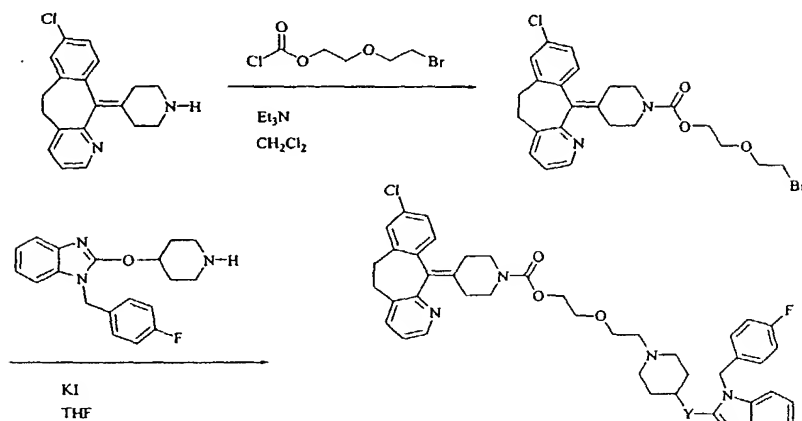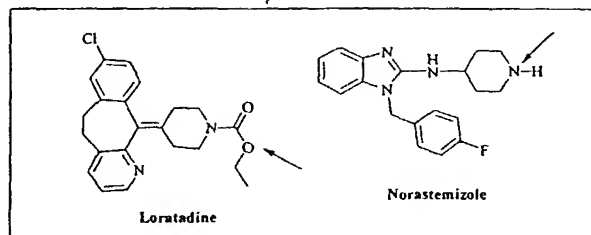
A solution of 2-bromoethyl ethylchloroformate (2 mmols) in CH_2Cl_2 (5 mL) containing Et_3N (0.2 mL) is stirred and cooled in an ice-water bath under an inert atmosphere. To this

5   is added dropwise a solution of the amine (CAS 100643-71-8; 2 mmols) in CH_2Cl_2 (5 mL). After addition is complete, the cooling bath is removed and the reaction solution is allowed to warm to room temperature. The progress of the reaction is followed by tlc and when reaction has occurred, the reaction solution is quenched in cold 5% aqueous Na_2CO_3. The layers are separated and the organic layer is washed with aqueous Na_2CO_3, with water and

10   is dried (Na_2SO_4), filtered and concentrated under reduced pressure to give the crude product. The desired compound is obtained by purification of the crude product with the use of HPLC.

A mixture of the product from the preceding reaction (1 mmols), 1-[(4-fluorophenyl)methyl]-N-(4-piperidinyl)-1H-benzimidazol-2-amine (Janssens, F.; et.al., J.

15   Med. Chem. 1985, 28, 1934-43; 1 mmols), and KI (1 mmols) in THF (10 mL) is stirrred under an inert atmosphere at RT. The progress of the reaction is monitored by TLC and when the reaction is complete, solvent is removed in vacuo. Water is mixed with the

- 236 -

residue and is extracted with CH$_2$Cl$_2$. The organic extract is washed with half saturated brine, dried (Na$_2$SO$_4$), filtered and concentrated under reduced pressure giving the crude product. The desired compound is obtained by purification of the crude product with the use of HPLC.

5

EXAMPLE A40

Loratadine

Citirizine
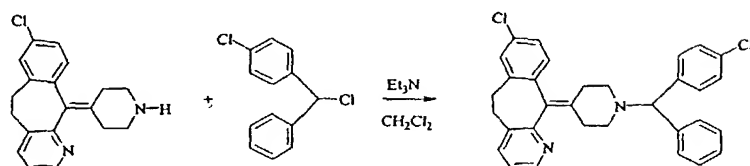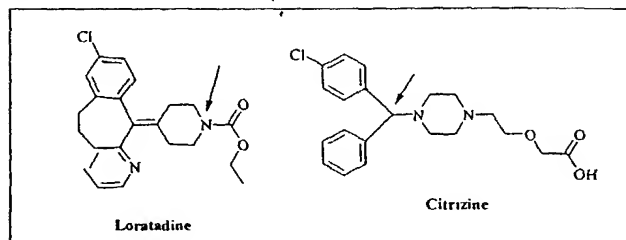
Et₃N
CH₂Cl₂

A solution of the amine (CAS 100643-71-8; 3 mmols) and 1-(4-chlorobenzhydryl)piperazine (CAS 303-26-4; 3 mmols) in methylene chloride (10 mL) and

5   Et₃N (0.3 mL) is stirred under an inert atmosphere. The solution is warmed and the course of the reaction is followed by TLC. When the reaction is complete, dil. aq. Na₂CO₃ is added to the solution, shaken, and the layers are separated. The aqueous layer is extracted with additional CH₂Cl₂, the combined organic extracts are washed with half-saturated brine, dried (Na₂SO₄), filtered and concentrated under reduced pressure giving the crude

10   product. The desired compound is obtained by purification of the crude product with the use of HPLC.

EXAMPLE A41
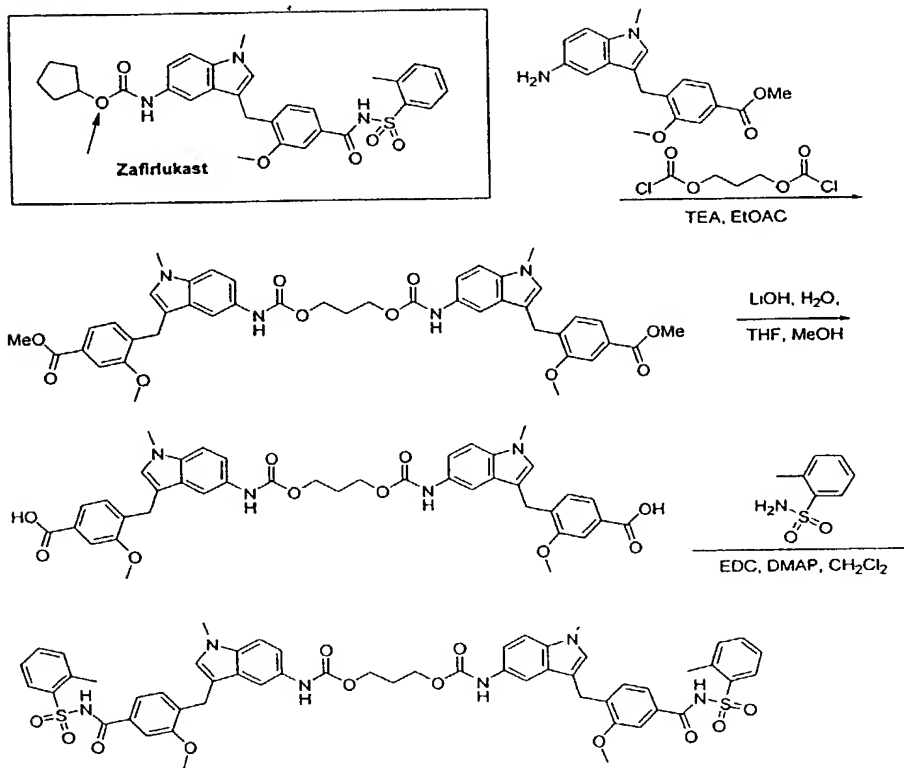
To a solution of 30 mmols of 4-[(5-amino-1-methyl-1H-indol-3-yl)methyl]-3-methoxy-, methyl ester (CAS 107754-14-3) in ethyl acetate with 30 mmols of triethylamine is added

5   15 mmols of pentanedioyl dichloride (CAS 2873-74-7). After 1h, the reaction is washed with water, dried over sodium sulfate, filtered and the solvent removed *in vacuo*. The residue is purified by chromatography to afford the desired product.

A solution of lithium hydroxide monohydrate (20 mmol) in water is added to a stirred solution of the above product (4.0 mmol) in a mixture of methanol and THF under

10   nitrogen. After 20 h, the mixture is concentrated in vacuo and acidified with 1 M hydrochloric acid. The white precipitate is collected by filtration, washed with a little water, and recrystallized from a toluene/hexanes mixture to afford the desired product.

A mixture of the above product (14.2 mmol), 2-methylbenzenesulfonamide (29.82 mmol), 4-(dimethylamino)pyridine (14.91 mmol), and 1-[3-(dimethylamino)propyl]-3-

15   ethylcarbodiimide hydrochloride (14.91 mmol) is dissolved in CH_2Cl_2, under nitrogen, and the mixture is stirred for 18 h. The mixture is then poured into 1 M HCl. The separated

aqueous layer is extracted with CH_2Cl_2, and the combined extracts are washed with water and brine, dried, and evaporated. The product is precipitated from hot methanol by water to afford the desired product.

EXAMPLE A42
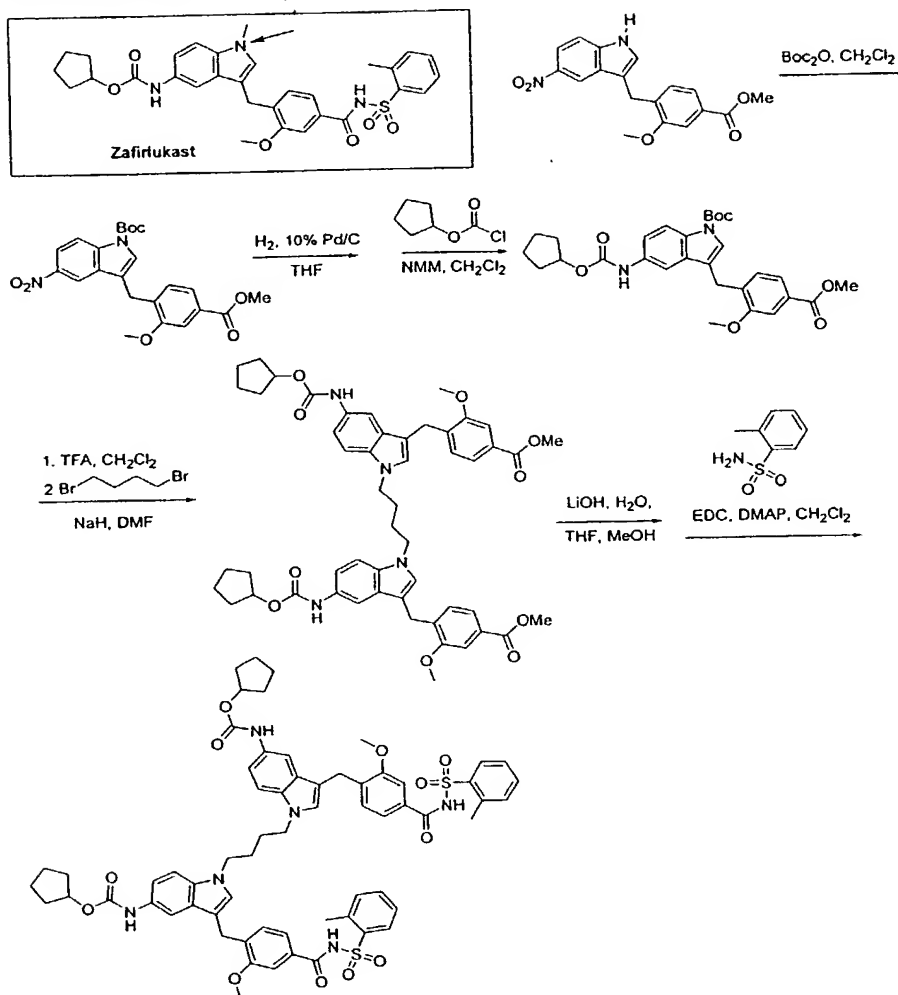
The starting compound (34.0 mmol), reported in J. Med. Chem. 1990, 33, 1781-1790,

is dissolved in dichloromethane under a nitrogen atmosphere. Di-tert-butyl dicarbonate

5 (Boc₂O) (119.12 mmol) dissolved in dichloromethane is added dropwise to the stirred

solution. . The course of the reaction is followed by TLC and stirring is continued at room

temperature until the reaction is judged complete. The reaction mixture is evaporated

giving a precipitate that is collected by filtration. The precipitate is rinsed with ether to

afford the desired product

10   Palladium-on-carbon (10% w/w) is added to a solution of the above product (30 mmol)

in THF and the mixture is hydrogenated at 3.45 bars for 2h. The mixture is filtered

through a pad of diatomaceous earth, and the solvent is evaporated. The product is purified by chromatography using ethyl acetate/hexanes as the eluant to afford the title product.

Cyclopentyl chloroformate (20 mmol) is added to a stirred solution of the above product (20 mmol) and N-methylmorpholine (20 mmol), in CH_2Cl_2 under nitrogen. The

5   mixture is stirred for 2h, then poured into 1 M hydrochloric acid, and extracted with ethyl acetate. The combined extracts are washed with saturated brine, died, and evaporated to give a viscous oil. The product is purified by chromatography, eluting with ethyl acetate/hexanes, to afford the title product.

The above product (15 mmol) is dissolved in CH_2Cl_2. A solution of 10%

10   trifluoroacetic acid in CH_2Cl_2 is added and the reaction is stirred for 1 hour at room temperature. The solvent is then removed *in vacuo* to provide the desired material as the TFA salt. The desired material is then purified from this mixture using HPLC. In the second step, the resulting amine (10 mmol) is added to a stirred suspension of oil-free sodium hydride (5.0 mmol) in dry THF, under nitrogen. After 10 min, 1,4-dibromobutane

15   (5.0 mmol) is added to the dark-red solution. After 30 min, the mixture is poured into 1 M hydrochloric acid and extracted with ethyl acetate. The combined extracts are washed with brine, then dried, and evaporated. The product is isolated by chromatography eluting with hexanes/CH_2Cl_2/ethyl acetate, to give a yellow oil, which is crystallized from a mixture of CH_2Cl_2 and hexanes to afford the title product.

20   A solution of lithium hydroxide monohydrate (50 mmol) in water is added to a stirred solution of the above product (10 mmol) in a mixture of methanol and THF under nitrogen. After 20 h, the mixture is concentrated in vacuo and acidified with 1 M hydrochloric acid. The white precipitate is collected by filtration, washed with a little water, and recrystallized from a toluene/hexanes mixture to afford the title product.

25   A mixture of the above product (5.0 mmol), 2-methylbenzenesulfonamide (10.5 mmol), 4-(dimethylamino)pyridine (5.25 mmol), and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (5.25 mmol) is dissolved in CH_2Cl_2, under nitrogen, and the mixture is stirred for 18 h. The mixture is then poured into 1 M HCl. The separated aqueous layer is extracted with CH_2Cl_2, and the combined extracts are washed with water

30   and brine, dried, and evaporated. The product is precipitated from hot methanol by water to afford the desired product.

EXAMPLE A43

Montelukast
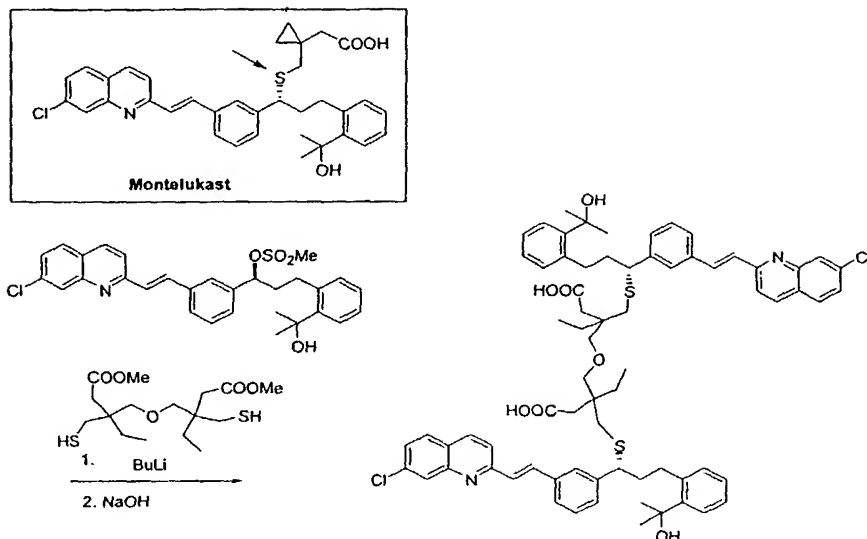
The starting compound is synthesized according to the procedure described in Drugs of the Future, 1997, 22(10), 1103-1111.

2-(2-Carboxy-2-mercaptomethyl-butoxymethyl)-2-mercaptomethyl-butyric acid is synthesized starting with di(trimethylolpropane), Registry Number 23235-61-2, according to the procedure described for the synthesis of 2-[1-(sulfanylmethyl)cyclopropyl]acetic acid in Drugs of the Future, 1997, 22(10), 1103-1111.

To 2-(2-Carboxy-2-mercaptomethyl-butoxymethyl)-2-mercaptomethyl-butyric acid (0.33 mmol) in degassed THF cooled at –15 °C is added slowly a solution of n-butyllithium (0.66mmol, 2.5 M in Hex) over 10 min. The heterogeneous mixture is warmed to –8 °C for 30 min. The mesylate, prepared in the first step, (0.66 mmol) in THF is added to the suspension and stirred at –15 °C overnight. Aqueous NH₄Cl is added and the mixture is extracted with ethyl acetate. After deprotection with NaOH, flash chromatography using Hex/EtOAc/AcOH as the eluant affords the desired product.
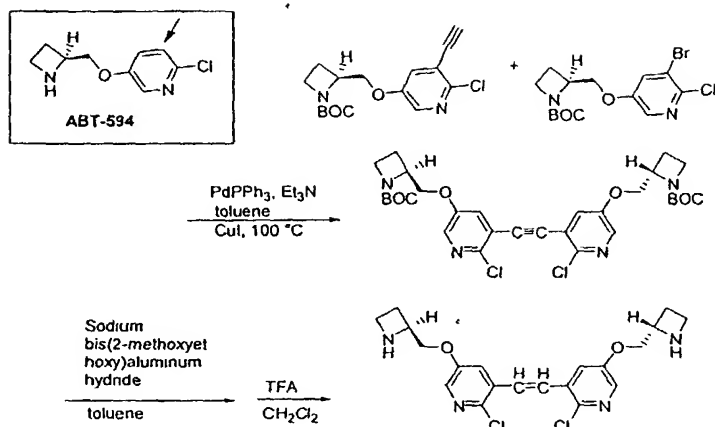
EXAMPLE A44

A solution of N-Boc-(S)-2-chloro-3-ethynyl-5-(2-azetidinylmethoxy)pyridine (1 mmol), prepared as described in PCT Application Publication No. WO 98/25920, N-Boc-(S)-2-chloro-3-bromo-5-(2-azetidinylmethoxy)pyridine (1 mmol), prepared as described in PCT Application Publication No. WO 98/25290, tetrakis(triphenylphosphine)palladium(0) (150 mg), and triethylamine (0.3 mL) in toluene (10 mL) is stirred with copper(I) iodide (20 mg). The mixture is stirred at 100 °C and the progress of the reaction is followed by tlc. After reaction is complete, the mixture is cooled, filtered, and the solvent is removed under reduced pressure to give the crude reaction product. The desired compound is obtained by purification of the crude product with the use of HPLC.

A solution of the above compound (1 mmol) in toluene (10 mL) is added dropwise to a stirred solution of sodium bis(2-methoxyethoxy)aluminum hydride (2 mmol) in toluene (10 mL) at 0 °C under an inert atmosphere. Stirring is continued at 0 °C and the progress of the reaction is followed by tlc. After the reaction is complete, the reaction mixture is added dropwise to vigorously stirred ice cold water (0.5 mL). Sodium potassium tartrate (10 mL, 0.5 M) is added and the reaction mixture is stirred for several hours. Ether is added to the reaction mixture and the layers are separated. The aqueous layer is further extracted with chloroform. The combined organic extracts are dried (Na$_2$SO$_4$), filtered and concentrated under reduced pressure to gibe the crude product. The desired compound is obtained by purification of the crude product with the use of HPLC.

A solution of the above compound and trifluoroacetic acid (3 mL) in CH_2Cl_2 (5 mL) is stirred at room temperature. The progress of the reaction is followed by tlc. After the reaction is complete, more CH_2Cl_2 is added and the solution is washed with aqueous Na_2CO_3 and with H_2O. The organic layer is dried (Na_2SO_4), filtered and concentrated
5   under reduced pressure to give the rude product. The desired compound is obtained by purification of the crude product with the use of HPLC.

EXAMPLE A45
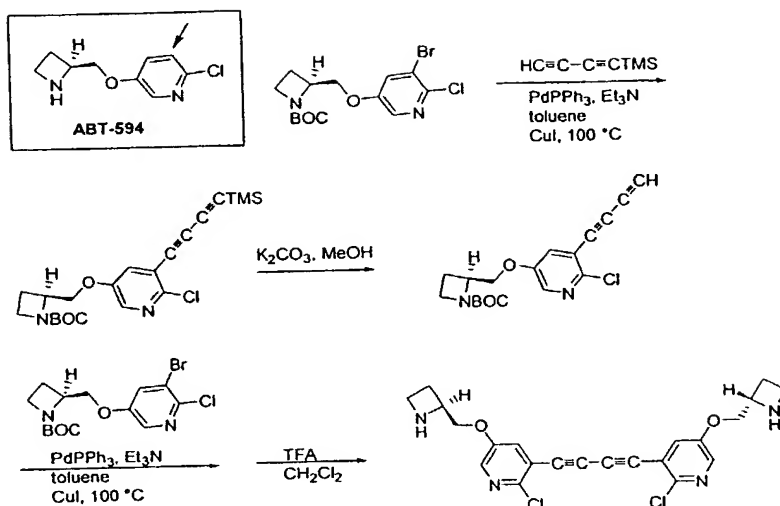
A solution of N-Boc- (S)-2-chloro-3-bromo-5-(2-azetidinylmethoxy)pyridine (1 mmol), tetrakis(triphenylphosphine)palladium(0) (100 mg), Buta-1,3-diynyl-trimethyl-silane (1mmol) and triethylamine (0.3 mL) in toluene (10 mL) is stirred with copper(I) iodide (15 mg). The reaction mixture is stirred at 100 °C and the progress of the reaction is followed by tlc. After the reaction is complete, the mixture is cooled, filtered, and the solvent is removed under reduced pressure to give the crude reaction product. The desired compound is obtained by purification of the crude product with the use of HPLC.

A solution of the above product in MeOH is stirred with K_2CO_3 and the progress of the reaction is followed by tlc. After the reaction is complete, the reaction mixture is filtered and the filtrated is concentrated under reduced pressure to give the crude product. The desired compound is obtained by purification of the crude product with the use of HPLC.

A solution of the above product (1 mmol), N-Boc- (S)-2-chloro-3-bromo-5-(2-azetidinylmethoxy)pyridine (1 mmol), tetrakis(triphenylphosphine)palladium(0) (150 mg), and triethylamine (0.3 mL) in toluene (10 mL) is stirred with copper(I) iodide (20 mg). The reaction mixture is stirred at 100 °C and the progress of the reaction is followed by tlc. After the reaction is complete, the mixture is cooled, filtered, and the solvent is removed under reduced pressure to give the crude reaction product. The desired compound is obtained by purification of the crude product with the use of HPLC.

- 246 -

A solution of the above product and trifluoroacetic acid (3 mL) in CH_2Cl_2 (5 mL) is
stirred at room temperature. The progress of the reaction is followed by tlc. After the
reaction is complete, more CH_2Cl_2 is added and the solution is washed with aqueous
Na_2CO_3 and H_2O. The organic layer is dried (Na_2SO_4), filtered and concentrated under
5   reduced pressure to give the crude reaction product. The desired compound is obtained by
purification of the crude product with the use of HPLC.

EXAMPLE A46
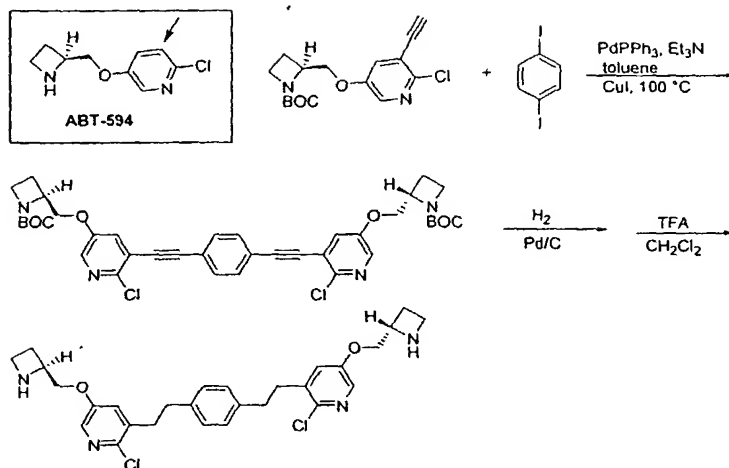
A solution of N-Boc- (S)-2-chloro-3-ethynyl-5-(2-azetidinylmethoxy)pyridine (2

mmol), 1,4-diiodobenzene (1 mmol), tetrakis(triphenylphosphine)palladium(0) (150 mg),

5   and triethylamine (0.3 mL) in toluene (10 mL) is stirred with copper(I) iodide (20 mg).

The reaction mixture is stirred at 100 °C and the progress of the reaction is followed by tlc.

·After the reaction is complete, the mixture is cooled, filtered, and the solvent is removed

under reduced pressure to give the crude reaction product. The desired compound is

obtained by purification of the crude product with the use of HPLC.

10   A solution of the above product (1 mmol) in ethyl acetate (10 mL) is hydrogenated at

atmospheric pressure in the presence of 10% palladium-on-carbon until tlc evidence shows

that reaction is complete. The reaction mixture is filtered through Celite® and the filter

pad is washed thoroughly with ethyl acetate. The combined filtrates are concentrated

under reduced pressure to gibe the crude product. The desired compound is obtained by

15   purification of the crude product with the use of HPLC.

A solution of the above product and trifluoroacetic acid (3 mL) in CH_2Cl_2 (5 mL) is

stirred at room temperature. The progress of the reaction is followed by tlc. After the

reaction is complete, more CH_2Cl_2 is added, more CH_2Cl_2 is added and the solution is

washed with aqueous Na_2CO_3 and H_2O. The organic layer is dried (Na_2SO_4), filtered and

20   concentrated under reduced pressure to give the crude reaction product. The desired

compound is obtained by purification of the crude product with the use of HPLC.
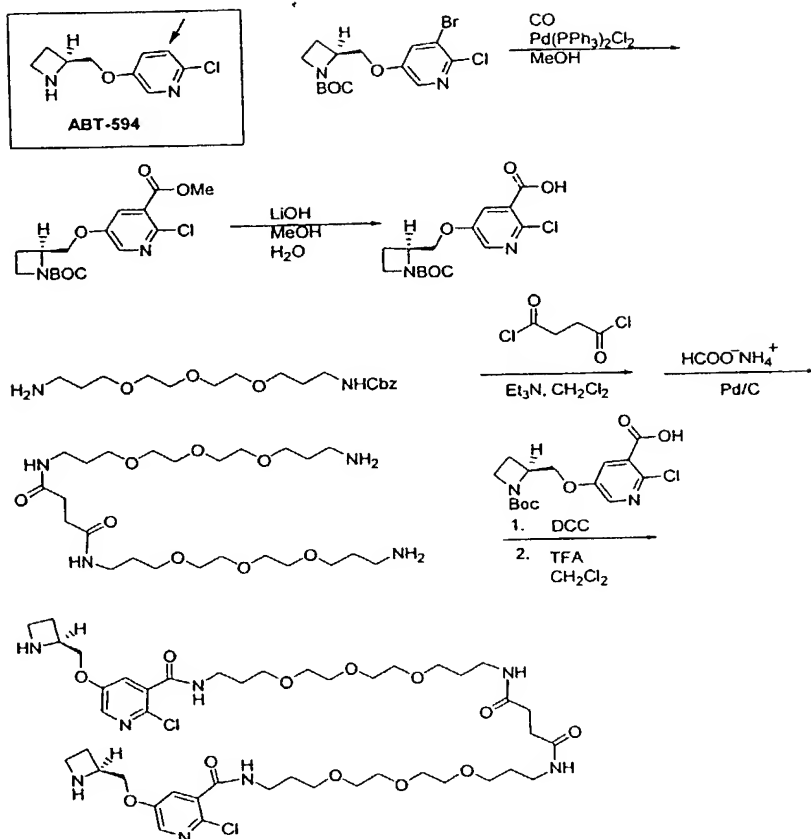
EXAMPLE A47
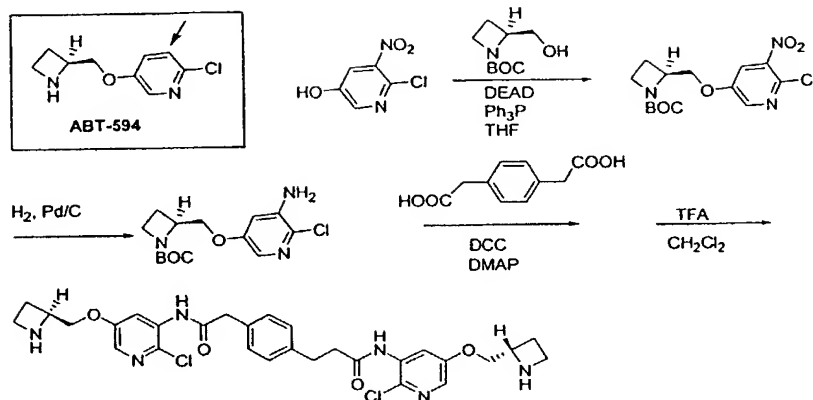
A solution of N-Boc-(S)-2-chloro-3-amino-5-(2-azetidinylmethoxy)pyridine (2 mmol) (prepared in the first two steps as shown above by known technique), benzene 1,3-bisacetic

5 acid (1 mmol) in CH_2Cl_2 (5 mL) is prepared under argon in a flask equipped with a magnetic stirrer and a drying tube. To this solution is added dicyclohexylcarbodiimide (solid, 2.2 mmol). The progress of the reaction is followed by tlc. After the reaction is complete, the reaction solution is quenched in water, aqueous sodium bicarbonate is added and the aqueous mixture is extracted with CH_2Cl_2. The organic layer is washed with

10 aqueous Na_2CO_3 and with H_2O, dried (Na_2SO_4), filtered and concentrated under reduced pressure to give the crude reaction product. The desired compound is obtained by purification of the crude product with the use of HPLC.
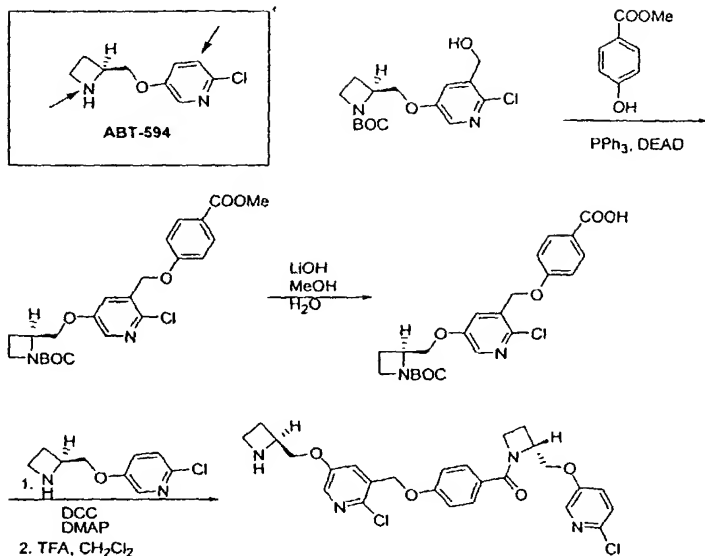
A solution of the above product and trifluoroacetic acid (3 mL) in CH_2Cl_2 (5 mL) is stirred at room temperature. The progress of the reaction is followed by tlc. After the

15 reaction is complete, more CH_2Cl_2 is added, more CH_2Cl_2 is added and the solution is washed with aqueous Na_2CO_3 and H_2O. The organic layer is dried (Na_2SO_4), filtered and concentrated under reduced pressure to give the crude reaction product. The desired compound is obtained by purification of the crude product with the use of HPLC.

EXAMPLE A48

A solution of the starting compound (10 mmol) (prepared in the first two steps as shown above, by conventional technique) in CH_2Cl_2 (10 mL) is added dropwise to a cooled

5 stirred solution of succinoyl chloride in CH_2Cl_2 (20 mL) and triethylamine (1 mL). The reaction mixture is allowed to come to room temperature and stirring is continued. Saturated aqueous potassium hydrogen sulfate is added carefully and the mixture stirred briefly. The layers are separated and the organic layer is washed with brine, dried (Na_2SO_4), filtered and concentrated under reduced pressure to give the crude reaction

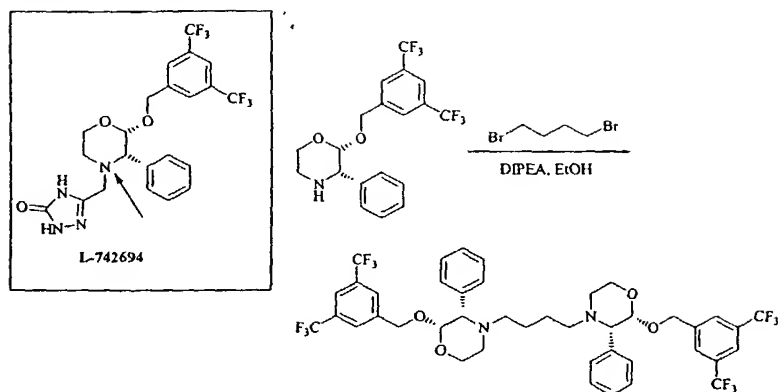10 product. The desired compound is obtained by purification of the crude product with the use of HPLC.

Ammonium formate (96 mg, 1.5 mmol) and 10% palladium-on-carbon (50 mg) are added to a solution of the product from the preceding reaction in methanol (3 mL) and THF (2 mL). The reaction mixture is stirred at 100 °C and the progress of the reaction is

monitored by tlc. After reaction is complete, the reaction mixture is filtered through Celite® and the filter pad is washed thoroughly with ethyl acetate. The combined organic layers are washed successively with aq. $NaHCO_3$ and with half-saturated brine, then filtered and concentrated under reduced pressure to gibe the crude product. The desired

5   compound is obtained by purification of the crude product with the use of HPLC.

The product from the preceding reaction (1 mmol) and N-Boc-(S)-2-chloro-3-carboxy-5-(2-azetidinylmethoxy)pyridine (2 mmol) in CH_2Cl_2 (5 mL) is prepared under argon in a flask equipped with a magnetic stirrer and a drying tube. To this solution is added dicyclohexylcarbodiimide (solid, 2.2 mmol). The progress of the reaction is followed by

10   tlc. After the reaction is complete, the reaction solution is quenched in water, aqueous sodium bicarbonate is added and the aqueous mixture is extracted with CH_2Cl_2. The organic layer is washed with aqueous Na_2CO_3 and with H_2O, dried (Na_2SO_4), filtered and concentrated under reduced pressure to give the crude reaction product. The desired compound is obtained by purification of the crude product with the use of HPLC.

15   A solution of the above product and trifluoroacetic acid (3 mL) in CH_2Cl_2 (5 mL) is stirred at room temperature. The progress of the reaction is followed by tlc. After the reaction is complete, more CH_2Cl_2 is added, more CH_2Cl_2 is added and the solution is washed with aqueous Na_2CO_3 and H_2O. The organic layer is dried (Na_2SO_4), filtered and concentrated under reduced pressure to give the crude reaction product. The desired

20   compound is obtained by purification of the crude product with the use of HPLC.

EXAMPLE A49

ABT-594

COOMe
HO
OH
PPh₃, DEAD

COOMe
NBOC
LiOH
MeOH
H₂O
COOH
NBOC

1. NH
DCC
DMAP
2. TFA, CH₂Cl₂

Diethyl azodicarboxylate (1 mmol) is added dropwise via syringe to a stirred solution of triphenylphosphine (1 mmol) in THF (5 mL) at room temperature. To this is added a

5   solution of N-Boc- (S)-2-chloro-3-hydroxymethyl-5-(2-azetidinylmethoxy)pyridine (1 mmol) and p-methoxycarbonylphenol (1 mmol) in THF (1 mL). The resulting solution is stirred at room temperature and the progress of the reaction is followed by tlc. After the reaction is complete, solvent is removed by evaporation under reduced pressure the residue is purified by HPLC to afford the desired compound.

10   A mixture of the product from the preceding reaction and lithium hydroxide (100 mmol) in methanol (6 mL) and H₂O (2 mL) is stirred at room temperature. The reaction is followed by tlc. After reaction occurs, the pH of the solution is adjusted to 7 by addition of dilute aq. Hydrochloric acid. The solvent is removed by lyophilization and the dry, crude product is used directly in the next step.

15   A solution of N-Boc- (S)-2-chloro-5-(2-azetidinylmethoxy)pyridine (1 mmol) and the product from the preceding reaction (1 mmol) in CH₂Cl₂ (5 mL) is prepared under argon in a flask equipped with a magnetic stirrer and a drying tube. To this solution is added dicyclohexylcarbodiimide (solid, 2.2 mmol). The progress of the reaction is followed by tlc. After the reaction is complete, the reaction solution is quenched in water, aqueous

20   sodium bicarbonate is added and the aqueous mixture is extracted with CH₂Cl₂. The

organic layer is washed with aqueous Na_2CO_3 and with H_2O, dried (Na_2SO_4), filtered and concentrated under reduced pressure to give the crude reaction product. The desired compound is obtained by purification of the crude product with the use of HPLC.

A solution of the above product and trifluoroacetic acid (3 mL) in CH_2Cl_2 (5 mL) is stirred at room temperature. The progress of the reaction is followed by tlc. After the reaction is complete, more CH_2Cl_2 is added, more CH_2Cl_2 is added and the solution is washed with aqueous Na_2CO_3 and H_2O. The organic layer is dried (Na_2SO_4), filtered and concentrated under reduced pressure to give the crude reaction product. The desired compound is obtained by purification of the crude product with the use of HPLC.

EXAMPLE A50

Under a nitrogen atmosphere, 5.00 mmols of the starting compound, described in EP-00577394, and 2.5 mmols of 1,4-dibromobutane are dissolved in EtOH in a sealed tube. DIPEA (2.75 mmols) is added, and the reaction is refluxed for 12 hours. The reaction is concentrated and the crude product is purified by silica-gel chromatography (MeOH/CH2Cl2). After concentration of the product-containing fractions, the product is dissolved in MeOH and 4 N HCl is added until the pH is between 1 and 2. The solution is stirred for 20 minutes and then pipetted into ether to precipitate the product as its dihydrochloride salt, which is then filtered and dried to yield the desired product.

EXAMPLE A51
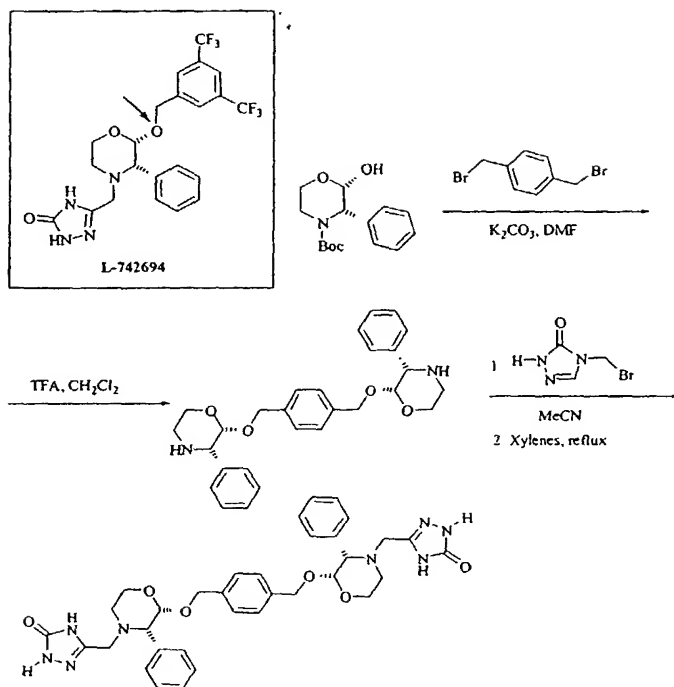
A solution of N-Boc-2-(S)-hydroxy-3-(S)-phenylmorpholine in DMF with 5.0 mmols of α,α'-dibromo-p-xylene and 10 mmols of potassium carbonate is heated as necessary and the reaction followed by TLC. When the reaction is judged complete, the mixture is partitioned between isopropyl acetate and water and the organic phase washed with water, dried over sodium sulfate and the solvent removed *in vacuo*. The residue is purified by chromatography to afford the desired product.

The above product (5 mmol) is dissolved in CH₂Cl₂. A solution of 10% trifluoroacetic acid in CH₂Cl₂ is added and the reaction is stirred for 1 hour at room temperature. The solvent is then removed *in vacuo* to provide the desired material as the TFA salt. The desired material is then purified from this mixture using reverse phase HPLC.

N-Methoxycarbonyl-2-chloroacetamidrazone (Yanagisawa, I.; Hirata, Y.; Ishii, Y. *J. Med. Chem.* 1984, 27, 849-857) is used as the alkylating agent and MeCN is used as the solvent. Flash chromatography on silica gel using CH₂Cl₂/MeOH/NH₄OH as the eluant affords 2-(S)-(3,5-Bis(trifluoromethyl)benzyloxy)-3-(S)-(4-iodophenyl)-4-(2-N-methoxy-carbonylacetamidrazono)morpholine.

A mixture of 2.2 mmol of 2-(S)-(3,5-Bis(trifluoromethyl)benzyloxy)-3-(S)-(4-iodophenyl)-4-(2-N-methoxy-carbonylacetamidrazono)morpholine in xylenes is heated at reflux. Dissolution of the solid occurs on warming. After 3 hours, the solution is cooled and concentrated *in vacuo*. Flash chromatography on silica gel using

5   CH_2Cl_2/MeOH/NH_4OH as the eluant affords the crude product. Recrystallization from hexanes/ethyl acetate affords the desired product.

EXAMPLE A52
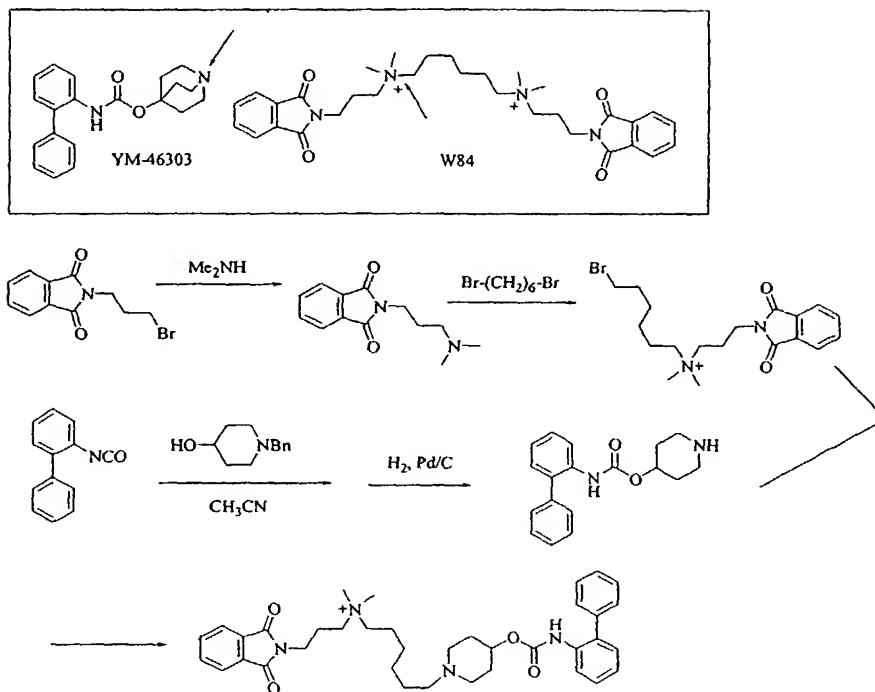
N-(3-bromopropyl)phthalimide (10 g, 37.3 mmol) was dissolved in dry acetonitrile (100 ml) and a solution of dimethylamine in tetrahydrofuran (56 ml, 111 mmol, 2 M) was

5   added. The flask was fitted with a reflux condense and the solution was heated at reflux. After 22 h, the reaction mixture was concentrated in vacuo to give a yellow oil which was partitioned between ethyl acetate and 1 M sodium carbonate solution saturated with sodium chloride. The organic phase was collected and washed with brine, dried over potassium carbonate, filtered and concentrated to give a yellow oil. The oil was dissolved in

10   methanol (25 ml) and p-toluenesulfonic acid (7.80 g, 41 mmol) was added. The solution was diluted with ether to crystallize the N-(3,3-dimethylaminopropyl)phthalimide as the p-toluenesulfonic acid salt (8.0 g). MS (M-OTs)$^+$ 233.1.

p-Toluenesulfonic acid salt of N-(3-dimethylaminopropyl)phthalimide (0.42 g, 0.98 mmol) was partitioned between ethyl acetate and 1 M sodium carbonate. The aqueous

15   phase was separated, saturated with sodium chloride and then extracted with ethyl acetate. The organic layers were washed with water and brine, dried over potassium carbonate, filtered and concentrated in vacuo to give an oil. The oil was dissolved in dry acetonitrile

(10 ml) and 1,6-dibromohexane (1.21 g, 4.90 mmol) was added. The reaction mixture was cooled to room temperature and diluted with one volume of ether. The resulting solids were filtered to give N-[N-(3,3-dimethyl)-N-(6-bromohexyl)aminopropyl)phthalimide quaternary ammonium salt as a white solid. MS 395.2 (M-Br)+.

In a 50 ml sealed tube was added 2-biphenylylisocyanate (8 g, 41 mmol) in 40 ml anhydrous acetonitrile. To this solution was added N-benzyl-4-piperidinol (9.8 g, 51.25 mmol) and the tube was partially immersed in a silicon oil bath and heated to 85 °C. After 16 h, the reaction mixture was cooled and concentrated in *vacuo* to give a 1-benzyl-4-piperidyl N-(2-biphenylyl)carbamate which was used in the next step without further purification.

1-Benzyl-4-piperidyl N-(2-biphenylyl)carbamate (12.5 g, 32.3 mmol) was dissolved in anhydrous methanol (150 ml) and formic acid (25 ml, 660 mmol) and the solution was flushed with gaseous nitrogen for 15 min. 10% Palladium on carbon (3 g) was added and the reaction mixture was stirred under nitrogen atmosphere . After 18 h, the reaction mixture was filtered through Celite and the filtrate was concentrated to give a yellow solid. The solid was partitioned between 0.1 N hydrochloric acid (300 ml) and diethyl ethr (300 ml). The aqueous layer was washed with diethyl ether and then basified with 1 N sodium hydroxide solution to pH 12. A white solid precipitated out which was extracted into ethyl acetate. The ethyl acetate layer was dried over magnesium sulfate and evaporated to dryness to give 4-piperidyl N-(2-biphenylyl)carbamate as a colorless solid (6.63 g, 69%). MS= 296.9 MH+.

To N-[N-(3,3-dimethyl)-N-(6-bromohexyl)aminopropyl)phthalimide quaternary ammonium salt (16 mg, 0.03 mmol) , prepared as above, in acetonitrile (1 ml) was added 4-piperidyl N-(2-biphenylyl)carbamate (10 mg, 0.03 mmol), prepared as above, and the reaction mixture was heated at reflux for 3 h. The reaction mixture was cooled to room temperature, and the product precipitated as the hydrobromide salt. The solids were isolated by filtration to give 20 mg (77%) of the desired product as white solids. The product was characterized by NMR (MeOH) and MS (calculated, $(M-HBr_2)^+ = 611.3600$; found, 611.5).

EXAMPLE A53
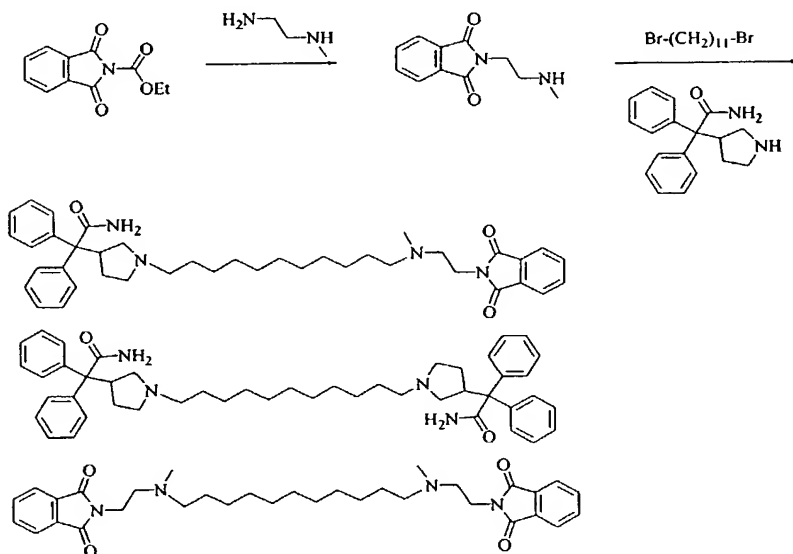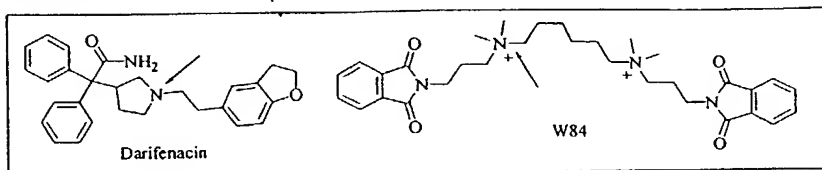
N-Methylethylenediamine (3.38 g, 45.6 mmol) was dissolved in chloroform (60 ml) and a solution of N-carbethoxyphthalimide (10 g, 45.6 mmol) in chloroform (30 ml) was added rapidly. After 6 h, the clear solution was concentrated in vacuo to give an oil which was dissolved in methanol, acidifed with 4 M hydrochloric acid in dioxane (15 ml). Diethyl ether was added to crystallize N-(2-methylaminoethyl)-phthalimido as the chloride salt (9.25 g, 84%). MS 205 (M-Cl)+

N-(2-Methylaminethyl)phthalimide (0.20 mL, of a 0.5 M solution, 0.10 mmol) (prepared by dissolving 168 mg of N-(2-methylaminethyl)phthalimido in, DIPEA (0.18 mL) and enough anhydrous acetonitrile to bring the solution to a total volume of 1.4 mL), and a solution of the darifenacin analog (0.167 mL) (prepared by dissolving 673 mg of the compound in enough anhydrous acetonitrile to bring the total volume to 4 mL), and NaI (0.20 mL of a 1 M solution in anhydrous acetonitrile) were combined in a 1 dram vial charged with 1,11-dibromoundecane (0.10 mmol). The vial was closed with a Teflon

sealed cap and the placed in a 72 C heating block for a 21 h. The mixture was cooled, quenched with 5% TFA/water (0.30 mL), diluted with acetonitrile and water, filtered, and purified using preperative LC/MS [Zeng, L; Kassel, D. B. *Anal. Chem.* 1998, *70*, 4380-4388 and references therein] to provide the desired compounds. Quality and identity of the collected fractions was verified using analytical HPLC and electrospray MS.
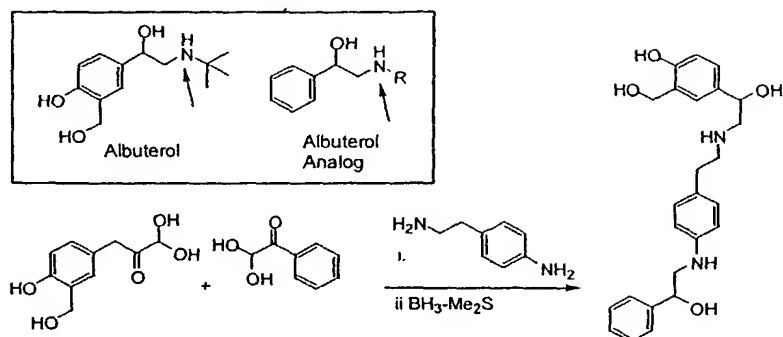
EXAMPLE A54
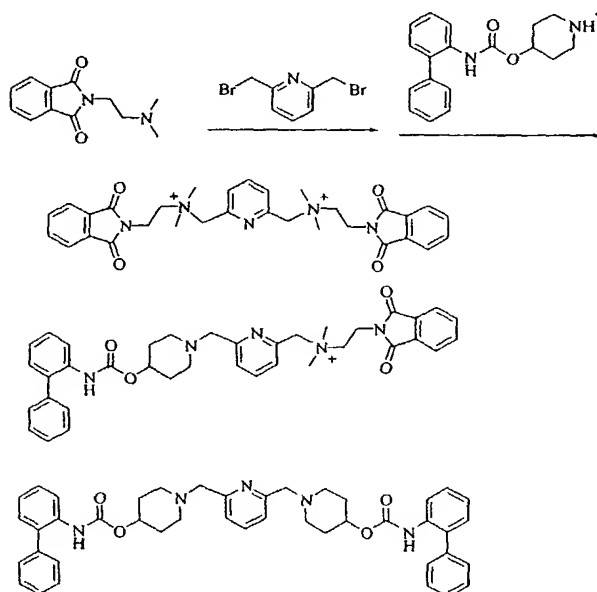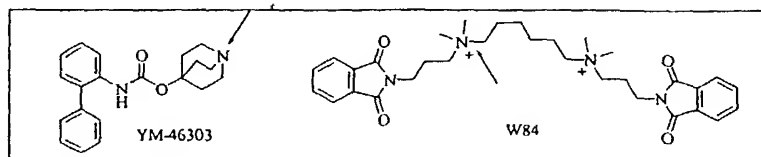
An aliquot (0.22 mL) of a solution of N-[2-dimethylamino)-ethyl]phthalimide (1.25 g,
3.2 mmol) and EtNiPr₂ (0.79 mL) dissolved in enough anhydrous acetonitrile to bring the

5   total volume up 6.4 mL was added to a 1 dram vial charged with 2,6-
bis(bromomethyl)pyridine (Aldrich, 26.5 mg, 0.10 mmol) in 0.22 mL of acetonitrile). The
vial was closed with a Teflon sealed cap and the placed in a 72 C heating block for a 24 h
to give a mixture of compounds. After cooling to room temperature, 4-piperidyl-N-(2-
biphenyl)-carbamate (0.33 mL) (prepared by dissolving 2.96 g in anhydrous DMF to

10   produce a total volume of 33 mL) was added and the vial is resealed and heated overnight
at 72 C in a heating block. The mixture was cooled, quenched with 5% TFA/water (0.30
mL), diluted with acetonitrile and water, filtered, and purified using preperative LC/MS [
Zeng, L; Kassel, D. B. *Anal. Chem.* 1998, *70*, 4380-4388 and references therein] to provide
the individual components. Quality and identity of the collected fractions was verified

15   using analytical HPLC and electrospray MS.

EXAMPLE A55
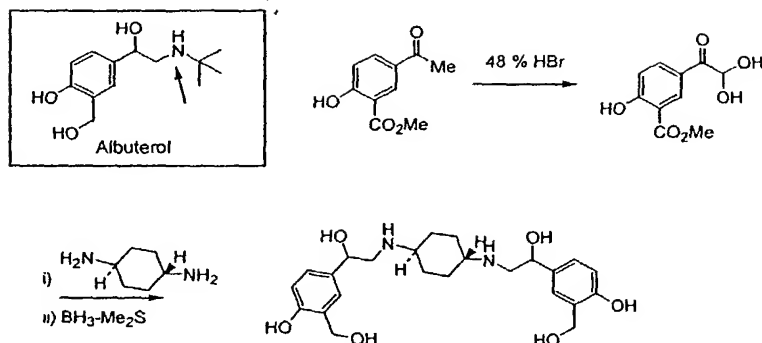
To a solution of 5-acetylsalicylic acid methyl ester (5.0g, 25.7 mmole) in dimethylsulfoxide (44 mL) was added 48% hydrobromic acid. The resulting mixture was

5 stirred at 55 °C for 24 h, and poured into a slurry of ice-water (~200 mL), precipitating a pale yellow solid. The solid was filtered, washed with water (200 mL), and dried to give α,α' dihydroxy-4-hydroxy-3-methoxycarbonyl-acetophenone. The product was re-suspended in ethyl ether (~200 mL), filtered and dried to give (3.41 g, 59%) of pure product. R_f = 0.8 (10% $MeOH/CH_2Cl_2$). H^1-NMR (4/1 $CDCl_3/CD_3OD$, 299.96 MHz): δ

10 (ppm) 8.73-8.72 (d, 1H), 8.28-8.24 (dd, 1H), 7.08-7.05 (d, 1H), 5.82 (s, 1H), 4.01 (s, 3H).

To a suspension of α, α'-dihydroxy-4-hydroxy-3-methoxycarbonyl-acetophenone (0.3 g, 1.33 mmole) in THF (10 mL) was added a solution of *trans*-1,4-diaminocyclohexane (76 mg, 0.66 mmole) in THF (5 mL). The resulting suspension was stirred for 3 h at ambient temperature under nitrogen atmosphere, at which formation of an imine was

15 completed judged by TLC analysis. After cooling of the resulting solution at ice bath, an excess amount of 2M BH_3-Me_2S in hexane (4 mL, 8 mmole) was added to the previous solution. The resulting mixture was slowly warmed to rt and refluxed for 4 h under N_2 stream. After cooling the reaction mixture, MeOH (5 mL) was added to quench excess amount of 2M BH_3-Me_2S. After stirring for 30 min., the final solution (or cloudy solution)

20 was evaporated *in vacuo*, yielding a pale brown solid. The solid was washed with EtOAc/hexane (1/2; 20 mL), and dried. The crude product was dissolved in 50% $MeCN/H_2O$ containing 0.5% TFA, and purified by prep-scale high performance liquid chromatography (HPLC) using a linear gradient (5% to 50% $MeCN/H_2O$ over 50 min, 20 mL/min; detection at 254 nM). Fractions with UV absorption were analyzed by LC-MS to

25 isolate *trans*-1,4-bis{N-[2-(4-hydroxy-3-hydroxymethyl-phenyl)-2-

hydroxyethyl]amino}cyclohexane. H^1-NMR (CD$_3$OD, 299.96 MHz): δ (ppm) 7.35 (d, 2H), 7.18 (dd, 2H), 6.80-6.78 (d, 2H), 4.88-4.86 (m, 2H), 4.65 (s, 4H), 3.15 (br s, 4H), 2.89 (m, 2H), 1.68-1.55 (br m, 4H); ESMS (C$_{24}$H$_{34}$N$_2$O$_6$): calcd. 446.5, obsd. 447.5 [M+H]$^+$.

EXAMPLE A56

To a suspension of α, α'-dihydroxy-4-hydroxy-3-methoxycarbonyl-acetophenone, prepared in Example A55 above, (0.3 g, 1.33 mmole) in THF (10 mL) was added a

5 solution of 2-(4-aminophenyl)ethylamine (0.181 g, 1.33 mmol) in THF (5 mL). The resulting suspension was stirred for 3 h at ambient temperature under nitrogen atmosphere, followed by addition of α, α'-dihydroxy-acetophenone (0.2g, 1.32 mmole). The reaction mixture was stirred for 3 h at RT, at which formation of the imine was completed as judged by TLC analysis. The reaction mixture was cooled in an ice bath and an excess amount of

10 2M BH_3-Me_2S in hexane (9 mL; 18 mmole) was added. The resulting mixture was slowly warmed to rt, and refluxed for 4 h under N_2 stream. After cooling, MeOH (10 mL) was added to quench excess amount of BH_3-Me_2S. After stirring 30 min., at rt, the final solution (or cloudy suspension) was evaporated *in vacuo*, to give a pale brown solid. The solid was washed with EtOAc/hexane (1/2; 20 mL), and dried. The crude product was

15 dissolved in 50% MeCN/H_2O containing 0.5% TFA, and purified by prep-scale high performance liquid chromatography (HPLC) using a linear gradient (5% to 50% MeCN/H_2O over 50 min, 20 mL/min; detection at 254 nM). Fractions with UV absorption were analyzed by LC-MS to locate 1-{2-[*N*-2-[(4-hydroxy-3-hydroxymethylphenyl)-2-hydroxyethyl]amino]-ethyl}-4-*N*-[2-phenyl-2-hydroxyethyl]amino]benzene. ESMS

20 ($C_{25}H_{30}N_2O_4$): calcd. 422.5, obsd. 423.3 $[M+H]^+$.

EXAMPLE A57
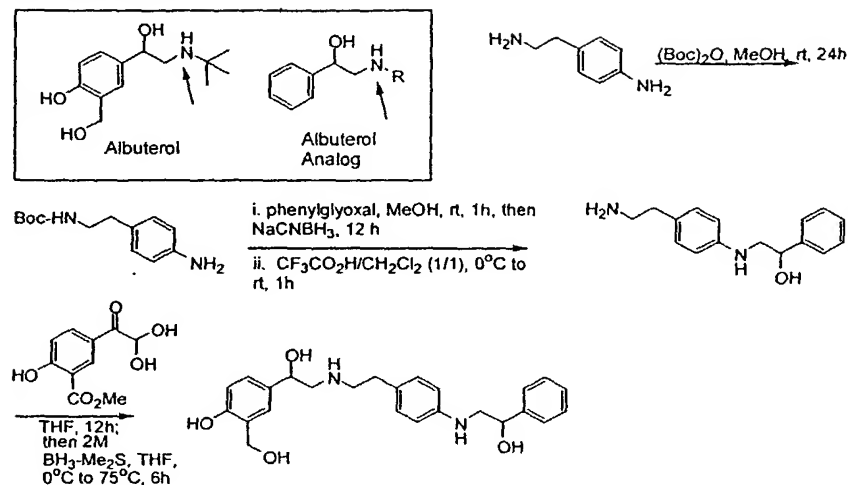
To a solution of 4-(2-aminoethyl)aniline (20 g, 147 mmole) in methanol (250 mL) was
added (Boc)$_2$O (32.4 g, 148 mmole) in methanol (50 mL) at rt. After stirring for 24 h, the
reaction mixture was concentrated to dryness to afford a pale yellow oily residue. The oily
material solidified slowly; thus it was dissolved in 5% MeOH/CH$_2$Cl$_2$, and subsequently
applied to flash silica column chromatography (3 to 10% MeOH/CH$_2$Cl$_2$). After
purification, 4-(N-Boc-2-aminoethyl)anilinewas obtained as a pale yellow solid (32.95g,
95%): R$_f$ = 0.6 in 10% MeOH/CH$_2$Cl$_2$. ^{1}H-NMR (CD$_3$OD, 299.96 MHz): δ (ppm) 6.96-
6.93 (d, 2H), 6.69-6.65 (d, 2H), 3.20-3.13 (q, 2H), 2.63-2.58 (t, 2H), 1.41 (s, 9H).

4-(N-Boc-2-aminoethyl)aniline (1.25 g, 5.29 mmole) was dissolved in methanol (30
mL), followed by addition of phenyl glyoxal (0.708 g, 5.28 mmole). The reaction mixture
was stirred for 1 h at rt, prior to addition of NaCNBH$_3$ (0.665 g, 10.6 mmole). The final
mixture was stirred for 12 h at rt, concentrated, and purified by flash silica column
chromatography (2 to 5% MeOH/CH$_2$Cl$_2$) to give N-(2-phenyl-2-hydroxyethyl)-4-(N-Boc-
2-aminoethyl)-aniline as a pale yellow oil (1.71 g, 91%): R$_f$ = 0.18 in 5% MeOH/CH$_2$Cl$_2$.
^{1}H-NMR (CD$_3$OD, 299.96 MHz): δ (ppm) 7.4-7.25 (m, 5H), 7.0-6.95 (d, 2H), 6.63-6.60 (d,
2H), 4.85-4.79 (dd, 1H), 3.3-3.21 (t, 2H), 3.2-3.15 (m, 2H), 2.64-2.5 (t, 2H), 1.42 (s, 9H).

A solution of N-(2-phenyl-2-hydroxyethyl)-4-(N-Boc-2-aminoethyl)aniline (1.7 g, 4.77
mmole) in methylene chloride (10 mL) was cooled in ice bath, and TFA (10 mL) was
slowly added under a stream of nitrogen gas. The reaction mixture was stirred for 1 h, and
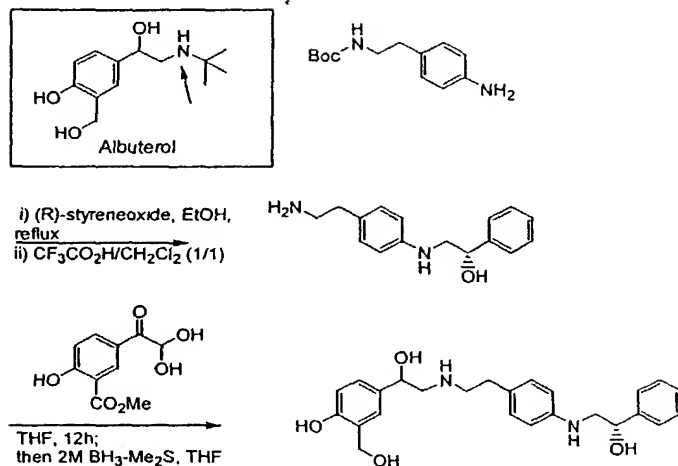
concentrated to yield a pale yellow oil. The crude material was purified by reversed phase HPLC (10% to 40% MeCN/H$_2$O over 50 min; 20 mL/min) to give N-(2-phenyl-2-hydroxyethyl)-4-(2-aminoethyl)aniline as the TFA salt (1.1 g). ^{1}H-NMR (CD$_3$OD, 299.96 MHz): δ (ppm) 7.42-7.3 (m, 5H), 7.29-7.25 (d, 2H), 7.12-7.0 (d, 2H), 4.85-4.82 (m, 1H),

5   3.45-3.35 (m, 2H), 3.18-3.1 (t, 2H), 2.98-2.94 (t, 2H); ESMS (C$_{16}$H$_{20}$N$_2$O$_1$): calcd. 256.4, obsd. 257.1 [M+H]$^+$, 278.8 [M+Na]$^+$, 513.4 [2M+H]$^+$.

To a solution of N-(2-phenyl-2-hydroxyethyl)-4-(2-aminoethyl)aniline trifluoroacetate salt (1.1 g, 2.3 mmole) in methanol (10 mL) was added 5 M NaOH solution (0.93 mL). After stirring for 10 min., the solution was concentrated to dryness. The residue was

10   dissolved in THF (25 mL), and α, α^c-dihydroxy-4-hydroxy-3-methoxy-carbonylacetophenone (0.514 g, 2.27 mmole), prepared in Example A55, was added. The reaction mixture was stirred for 12 h at rt, cooled to 0 °C, and BH$_3$/Me$_2$S (1.14 mL, 10 M) was added under nitrogen atmosphere. The reaction mixture was gradually warmed to rt, stirred for 2 h at rt, and refluxed for 4 h. The reaction mixture was cooled and methanol

15   (10 mL) was added slowly. After stirring for 30 min., at rt, the reaction mixture was concentrated to afford a solid residue, which was dissolved in MeOH (20 mL) containing 10% TFA. Evaporation of the organics yielded a pale yellow oil which was purified by reversed phase HPLC: 10% to 30% MeCN/H$_2$O over 50 min; 20 mL/min to give 1-{2-[N-2-(4-hydroxy-3-hydroxy-methylphenyl)-2-hydroxyethyl]-amino]ethyl}-4-[N-(2-phenyl-2-

20   hydroxyethyl)-amino]benzene as the TFA salt (0.65 g). ^{1}H-NMR (CD$_3$OD, 299.96 MHz): δ (ppm) 7.42-7.3 (m, 6H), 7.28-7.24 (d, 2H), 7.18-7.14 (dd, 1H), 7.1-7.07 (d, 2H), 6.80-6.77 (d, 1H), 4.86-4.82 (m, 2H), 4.65 (s, 2H), 3.44-3.34 (m, 2H), 3.28-3.22 (m, 2H), 3.20-3.14 (m, 2H), 3.04-2.96 (m, 2H); ESMS (C$_{25}$H$_{30}$N$_2$O$_4$): calcd. 422.5, obsd. 423.1 [M+H]$^+$, 404.7 [M-1H$_2$O]$^+$, 387.1 [M-2H$_2$O]$^+$.

EXAMPLE A58

i) (R)-styreneoxide, EtOH, reflux
ii) CF₃CO₂H/CH₂Cl₂ (1/1)

THF, 12h;
then 2M BH₃-Me₂S, THF

A solution of 4-(N-Boc-2-aminoethyl)aniline (7.0 g, 29.6 mmole), prepared Example A57, in ethanol (100 mL) and (R)-styreneoxide (3.56 g, 29.6 mmole) was refluxed for 24 h. The organics were removed to give a pale yellow solid. N-(2-phenyl-2-(S)-hydroxyethyl)-4-(N-Boc-2-aminoethyl)aniline was separated by flash silica column chromatography: 1/2 EtOAc/hexane to 3/1 EtOAc/hexane to 3% MeOH in 3/1 EtOAc/hexane: Rf = 0.39 in 3% MeOH/CH₂Cl₂.

A solution of N-(2-phenyl-2-(S)-hydroxyethyl)-4-(N-Boc-2-aminoethyl)-aniline (2.5 g, 7.0 mmole) in CH₂Cl₂ (15 mL) was cooled in an ice bath under stream of nitrogen and TFA (15 mL) was slowly added. The reaction mixture was stirred for 2 h at 0°C and then concentrated in vacuo. The crude product was dissolved in 20% MeCN/H₂O and purified by preparative reversed phase HPLC (5 to 2% MeCN/H₂O over 50 min; 254 nm; 20 mL/min.), to give N-(2-phenyl-2-(S)-hydroxyethyl)-4-(2-aminoethyl)aniline trifluoroacetate salt as a colorless oil. ¹H-NMR (CD₃OD, 299.96 MHz): δ (ppm); 7.45-7.25 (m, 9H), 4.9 (dd, 1H), 3.55-3.45 (m, 2H), 3.21-3.15 (t, 2H), 3.05-2.95 (t, 2H) ESMS (C₁₆H₂₀N₂O₁): calcd. 256.4, obsd. 257.1 [M+H]⁺, 280.2 [M+Na]⁺.

To a solution of N-(2-phenyl-2-(S)-hydroxyethyl)-4-(2-aminoethyl)aniline trifluoroacetate (0.144 g, 0.3 mmole) in methanol (10 mL) was added aq. NaOH solution (1.0 M, 0.625 mL). The solution was concentrated to dryness and the residue was dissolved in anhydrous THF (5 mL). α, α'-Dihydroxy-4-hydroxy-3-methoxycarbonylacetophenone (0.067 g, 0.3 mmole), prepared in Example A55, was

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added and the reaction mixture was stirred for 12 h at rt. BH$_3$-Me$_2$S (0.2 mL, 2M) was added at 0°C and the reaction mixture was heated at 75 °C for 6 h. After cooling the reaction mixture in ice bath, MeOH (5 mL) was slowly added to it to quench the reaction, and the reaction mixture was stirred for 30 min., at rt. The organics were removed and the residue was dissolved in TFA/MeOH (1/9; 20 mL), and concentrated. The crude product was dissolved in 20% MeCN/H$_2$O, and purified by preparative HPLC: 5 to 20% MeCN/H$_2$O; 20 mL/min; 254 nm.) to give 1-{2-[N-2-(4-hydroxy-3-hydroxy-methylphenyl)-2-hydroxyethyl]amino]ethyl}-4-[N-(2-phenyl-2-(S)-hydroxyethyl)-amino]benzene. ^{1}H-NMR (CD$_3$OD, 299.96 MHz): δ (ppm) 7.42-7.29 (m, 8H), 7.22-7.18 (d, 2H), 7.17-7.14 (dd, 1H), 6.80-6.77 (d, 1H), 4.9-4.85 (m, 2H), 4.65 (s, 2H), 3.5-3.34 (m, 2H), 3.28-3.25 (m, 2H), 3.19-3.14 (m, 2H), 3.04-2.98 (m, 2H); ESMS (C$_{25}$H$_{30}$N$_2$O$_4$): calcd. 422.5, obsd. 423.1 [M+H]$^+$, 446.1 [M+Na]$^+$.

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EXAMPLE A59
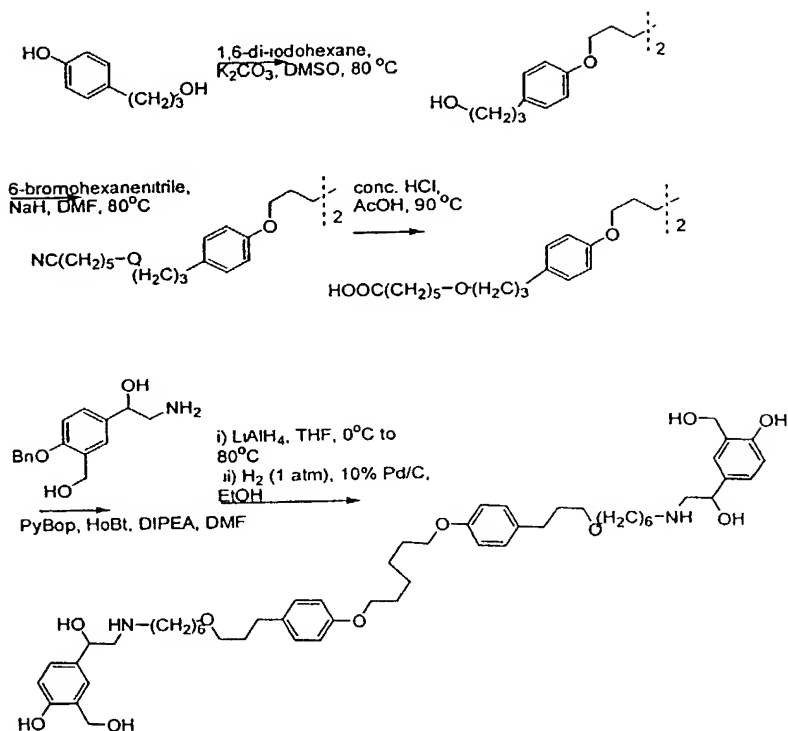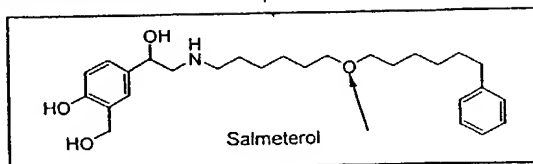
A solution of 3-(4-hydroxyphenyl)-1-propanol (3.3 g, 21.7 mmole) and 1,6-di-iodohexane (3.5 g, 8.88 mmole) in dimethylsulfoxide (40 mL) was degassed and saturated

5 with N$_2$ gas and potassium carbonate (4.5 g, 32.56 mmole) was added. The reaction mixture was stirred at 80 °C for 18 h under nitrogen atmosphere and then quenched with brine (150 mL). The product was extracted with EtOAc (200 mL) and the organic extracts were washed with 0.1 M NaOH and brine, and dried with MgSO$_4$. The organics were removed *in vacuo* to give a pale brown solid. The solid was purified by flash silica column

10 chromatography: 4/1 hexane/EtOAc to 5% MeOH in 1/1 hexane/EtOAc to give 1,6-bis[4-(3-hydroxypropyl)phenoxy]hexane (R$_f$ = 0.17 in 1/1 hexane/EtOAc) in 65% yield (2.23 g). ^{1}H-NMR (CD$_3$OD, 299.96 MHz): δ (ppm) 7.08-7.05 (d, 4H), 6.80-6.77 (d, 4H), 3.93-3.89 (t, 4H), 3.56-3.52 (t, 4H), 2.64-2.56 (t, 4H), 1.81-1.69 (m, 8H), 1.44-1.21 (m, 4H).

A solution of 1,6-bis[4-(3-hydroxypropyl)phenoxy]hexane (2.2 g, 5.69 mmole) in DMF (10 mL) was added to a solution of DMF (40 mL) containing NaH (0.57 g; 60% dispersion in mineral oil) at 0 °C under nitrogen atmosphere and the reaction mixture was heated at 50 °C. After 1 h, 6-bromohexanenitrile (2.26 mL, 17 mmole) was added and the

5 reaction mixture was heated at 80 °C for 24 h. The reaction mixture was quenched with brine solution (100 mL) and was extracted with EtOAc (250 mL). The organic phase was washed with brine, dried with MgSO$_4$, and evaporated *in vacuo*, to give a pale yellow oil. Purification by flash silica column chromatography: 4/1 to 1/1 hexane/EtOAc afforded 1,6-bis[4-(5-cyanopentyloxypropyl)]phenoxy]hexane product (R$_f$ = 0.6 in 1/1 EtOAc/hexane).

10 ^{1}H-NMR (CDCl$_3$, 299.96 MHz): δ (ppm) 7.09-7.06 (d, 4H), 6.82-6.79 (d, 4H), 3.94-3.90 (t, 4H), 3.42-3.37 (m, 8H), 2.64-2.58 (t, 4H), 2.40-2.32 (m, 8H), 1.90-1.26 (m, 24H).

The 1,6-bis[4-(5-cyanopentyloxypropyl)]phenoxy]hexane (0.278 g, 0.48 mmole) obtained in Step 2 above was added to a mixture of conc. HCl (10 mL) and AcOH (2 mL) and the reaction mixture was heated at 90 °C. After 15 h, the reaction mixture was diluted

15 with brine (50 mL), extracted with EtOAc (100 mL), and dried with MgSO$_4$. Evaporation of the organic phase afforded the 1,6-bis[4-(5-carboxypentyl-oxypropyl)]phenoxy]hexane as a pale yellow oily residue, which was used in next step without further purification. ^{1}H-NMR (CDCl$_3$, 299.96 MHz): δ (ppm) 7.09-7.07 (d, 4H), 6.82-6.79 (d, 4H), 3.96-3.92 (t, 4H), 3.42-3.56 (m, 8H), 2.64-2.59 (t, 4H), 2.39-2.32 (m, 4H), 1.91-1.40 (m, 24H).

20 To a solution of 2-hydroxy-2-(4-benzyloxy-3-hydroxymethylphenyl)-ethylamine (0.263 g, 0.96 mmole) in DMF (8 mL) was added 1,6-bis[4-(5-carboxypentyloxypropyl)phenoxy]hexane (~0.48 mmole), obtained in Step 3 above, HOBt (0.13 g, 0.96 mmole), DIPEA (0.21 mL, 1.20 mmole), and PyBOP (0.502 g, 0.96 mmole). After stirring for 24 h at rt, the reaction mixture was diluted with brine (20 mL) and

25 extracted with EtOAc (50 mL). The organic layer was washed with 0.1 M NaOH, 0.1 M HCl, and brine, and dried over MgSO$_4$. The organic solvents were removed *in vacuo* to give 1,6-bis[4-(5-amidopentyloxypropyl)-phenoxy]hexane as a pale yellow oily residue (0.45 g).

A solution of 1,6-bis[4-(5-amidopentyloxypropyl)-phenoxy]hexane (0.45 g, 0.4

30 mmole) obtained in Step 4 above, in anhydrous THF (10 mL) was added to a solution of LiAlH$_4$ (0.16 g, 4.22 mmole) in anhydrous THF (40 mL) at 0°C . The reaction mixture was stirred for 4 h at 80 °C under nitrogen atmosphere and then quenched by with 10%

NaOH (1 mL) at 0 °C. After 30 min., the reaction mixture was filtered and the precipitate was washed with 10% MeOH in THF (50 mL). The filtrates were combined and evaporated *in vacuo* to give a pale yellow oily residue. Purification by flash silica column chromatography: 5% MeOH/CH₂Cl₂ to 3% *i*-PrNH₂ in 10% MeOH/CH₂Cl₂ gave the 1,6-bis[4-(6-aminohexyloxypropyl)-phenoxy]hexane. ^{1}H-NMR (CDCl₃, 299.96 MHz): δ (ppm) 7.40-7.25 (m, 12H), 7.22-7.18 (d, 2H), 7.09-7.02 (d, 4H), 6.91-6.88 (d, 2H), 6.81-6.75 (d, 4H), 5.01 (s, 4H), 4.8-4.75 (m, 2H), 4.70 (s, 4H), 3.96-3.83 (q, 4H), 3.42-3.34 (m, 8H), 2.84-2.64 (m, 8H), 2.62-2.56 (t, 4H), 1.84-1.75 (m, 8H), 1.57-1.50 (m, 10H), 1.34-1.23 (m, 10H).

A solution of 1,6-bis[4-(6-aminohexyloxypropyl)-phenoxy]hexane (0.16 g, 0.15 mmole) obtained in Step 5 above, in EtOH (40 mL) was hydrogenated under H₂ (1 atm) atmosphere with 10% Pd/C catalyst (100 mg) at rt for 24 h. The catalyst was filtered and the filtrate was concentrated to afford crude product as a pale yellow oil. Purification by reversed phase HPLC: 10 to 50% MeCN/H₂O over 40 min; 20 mL/min; 254 nm provides 1,6-bis{4-(*N*-[2-(4-hydroxy-3-hydroxymethyl-phenyl)-2-hydroxyethyl]aminohexyloxypropyl]-phenoxy}hexane. H^1-NMR (CD₃OD, 299.96 MHz): δ (ppm) 7.35 (d, 2H), 7.18-7.15 (dd, 2H), 7.08-7.05 (d, 4H), 6.82-6.77 (m, 6H), 4.65 (s, 4H), 3.96-3.92 (t, 4H), 3.45-3.34 (m, 8H), 3.12-3.01 (m, 6H), 2.94-2.89 (t, 2H), 2.62-2.57 (t, 4H), 1.86-1.43 (m, 28H); ESMS (C₅₄H₈₀N₂O₁₀): calcd. 917.1, obsd. 917.5 [M]⁺, 940.8 [M+Na]⁺.
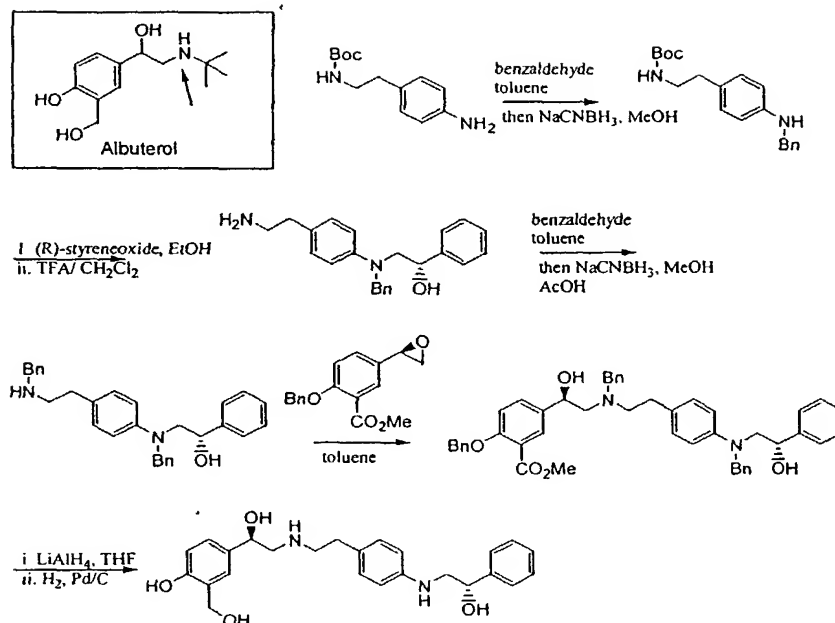
EXAMPLE A60

A mixture of 4-(N-Boc-2-aminoethyl)aniline (10 g, 42.34 mmole), prepared in Example A57, benzaldehyde (4.52 mL, 44.47 mmole), and molecular sieves 4A (10 g) in toluene (100 mL) was refluxed at 95 °C for 15 h. The reaction mixture was filtered and the filtrate was concentrated *in vacuo* to give a colorless oil. The oil was dissolved in MeOH (150 mL) and AcOH (0.5 mL) and NaCNBH$_3$ (2.79 g, 44.4 mmole) were added. The reaction mixture was stirred at 0 °C for 1 h and at rt for 2 h and then concentrated *in vacuo* to give a pale yellow oily residue. Purification by flash silica column chromatography: 1/1 hexane/EtOAc gave N-benzyl-4-(N-Boc-2-aminoethyl)aniline as colorless oil (11.5 g, 83%). R$_f$ = 0.75 in 1/1 hexane/EtOAc. H^1-NMR (CD$_3$OD, 299.96 MHz): δ (ppm) 7.38-7.2 (m, 5H), 6.87-6.84 (d, 2H), 6.58-6.55 (d, 2H), 4.27 (s, 2H), 3.2-3.15 (m, 2H), 2.6-2.56 (t, 2H), 1.41 (s, 9H); ESMS (C$_{20}$H$_{26}$N$_2$O$_2$): calcd. 326.4, obsd. 328 [M+H]$^+$.

A mixture of N-benzyl-4-(N-Boc-2-aminoethyl)aniline (10 g, 30.7 mmole) and (R)-styreneoxide (3.51 mL, 30.7 mmole) in EtOH (100 mL) was refluxed for 48 h. A small aliquot of the reaction mixture was taken out for liquid chromatographic analysis, which indicated that the desired adduct 2-[(N-benzyl-4-[2-N-Boc-aminoethyl)anilino]-1-phenylethanol was formed as a minor product along with another regio-isomer 2-[(N-benzyl-4-[2-N-Boc-aminoethyl)anilino]-2-phenyl-ethanol in a ratio of ~1/2. Evaporation

of the solution afforded thick, pale yellow oil, which was purified by flash silica column

chromatography: 4/1 to 2/1 hexane/EtOAc. After repeated chromatography, 2-[(N-benzyl-

4-[2-N-Boc-aminoethyl)anilino]-1-phenyl-ethanol was obtained as a colorless oil (4.01 g,

29%) (R$_f$ = 0.76 in 2/1 hexane/EtOAc). H^1-NMR (CD$_3$OD, 299.96 MHz): δ (ppm) 7.4-7.1

5   (m, 10H), 7.1-7.06 (d, 2H), 6.68-6.65 (d, 2H), 5.0 (t, 1H), 4.52-4.46 (d, 1H), 4.26-4.22 (d,

1H), 3.76-3.68 (dd, 1H), 3.56-3.48 (dd, 1H), 3.22-3.12 (m, 2H), 2.68-2.56 (m, 2H), 1.41 (s,

9H); ESMS (C$_{28}$H$_{34}$N$_2$O$_3$): calcd. 446.6, obsd. 447.1 [M+H]$^+$, 893.4 [2M+H]$^+$.

To a solution of 2-[(N-benzyl-4-[2-N-Boc-aminoethyl)anilino]-1-phenyl-ethanol

(4.01 g, 8.99 mmole) in CH$_2$Cl$_2$ (15 mL) maintained in an ice bath was added TFA (15

10   mL) under stream of nitrogen atmosphere. After stirring at 0 °C for 30 min., the reaction

mixture was concentrated *in vacuo*, yielding a pale yellow oil. Purification by flash silica

column chromatography: (½ hexane/EtOAc to 5% i-PrNH$_2$ in ½ hexane/EtOAc) gave 2-

[(N-benzyl-4-[2-aminoethyl)anilino]-1-phenyl-ethanol as a pale yellow oil from such

fractions with R$_f$ of 0.2 (5% i-PrNH$_2$ in ½ hexane/EtOAc) in 74% yield (2.29 g). H^1-NMR

15   (CD$_3$OD, 299.96 MHz): δ (ppm) 7.38-7.06 (m, 10H), 7.01-6.98 (d, 2H), 6.71-6.68 (d, 2H),

5.02-4.96 (dd, 1H), 4.54-4.48 (d, 1H), 4.29-4.23 (d, 1H), 3.76-3.67 (dd, 1H), 3.58-3.50 (dd,

1H), 2.82-2.74 (t, 2H), 2.64-2.59 (t, 2H); ESMS (C$_{23}$H$_{26}$N$_2$O$_1$): calcd. 346.5, obsd.

346.3[M]$^+$,

A mixture of 2-[(N-benzyl-4-[2-aminoethyl)anilino]-1-phenylethanol (2.28 g, 6.59

20   mmole), benzaldehyde (0.74 mL, 7.28 mmole), and molecular sieves 4A (4 g) in toluene

(40 mL) was heated at 90 °C for 14 h. The reaction mixture was cooled and filtered, and

the sieves were rinsed with toluene. The combined filtrates were concentrated to give an

oily residue which was washed with hexane, and dried. The residue was dissolved in

MeOH (40 mL) containing AcOH (0.4 mL) and the reaction mixture was cooled in an ice

25   bath. NaCNBH$_3$ (0.62 g, 9.87 mmole) was added and the reaction mixture was stirred for 2

h at rt, and then concentrated. The oily residue was dissolved in 60% MeCN/H$_2$O, and

purified by reversed phase preparative liquid chromatography (40 to 80% MeCN/H$_2$O over

30 min; 30 mL/min) to give 2-[(N-benzyl-4-[2-N-benzylaminoethyl)anilino]-1-

phenylethanol as the TFA salt. The product was treated with alkaline brine solution, and

30   extracted with ether (200 mL). The organic layer was dried with NaSO$_4$, and concentrated,

to give 2-[(N-benzyl-4-[2-N-benzylaminoethyl)anilino]-1-phenylethanol as a colorless oil

(1.36 g). H^1-NMR (CD$_3$OD, 299.96 MHz): δ (ppm) 7.36-7.06 (m, 15H), 6.98-6.95 (d, 2H),

6.69-6.60 (d, 2H), 5.01-4.96 (t, 1H), 4.54-4.47 (d, 1H), 4.29-4.24 (d, 1H), 3.73 (s, 2H),

3.72-3.68 (dd, 1H), 3.59-3.54 (dd, 1H), 2.80-2.74 (m, 2H), 2.70-2.64 (m, 2H); ESMS

($C_{30}H_{32}N_2O_1$): calcd. 436.6, obsd. 437.2 [M+H]$^+$.

A concentrated solution of 2-[(N-benzyl-4-[2-N-benzylaminoethyl)anilino]-1-

5   phenylethanol (1.36 g, 3.12 mmole) and compound (S)-4-benzyloxy-3-

methoxycarbonylstyreneoxide (0.887 g, 3.12 mmole; ~95% ee) (prepared as described in

R. Hett, R. Stare, P. Helquist, *Tet. Lett., 35,* 9375-9378, (1994)) in toluene (1 mL) was

heated at 105 °C for 72 h under nitrogen atmosphere. The reaction mixture was purified by

flash silica column chromatography (2/1 hexane/EtOAc to 3% MeOH in 1/1

10   hexane/EtOAc) to give 1-{2-[N-benzyl-N-2-(4-benzyloxy-3-methoxycarbonylphenyl)-2-

(R)-hydroxy]ethylaminoethyl}-4-[N-(2-phenyl-2-(S)-hydroxy)ethylamino]benzene. (R_f =

0.62 in 3% MeOH in 1/1 hexane/EtOAc) was obtained as a pale yellow foam (2.0 g, 89%).

H^1-NMR (CD$_3$OD, 299.96 MHz): δ (ppm) 7.67-7.66 (d, 1H), 7.49-7.42 (m, 2H), 7.38-7.0

(m, 20H), 6.88-6.85 (d, 2H), 6.65-6.62 (d, 2H), 5.15 (s, 2H), 5.05-4.98 (t, 1H), 4.6-4.54 (t,

15   1H), 4.53-4.46 (d, 1H), 4.28-4.22 (d, 1H), 3.84 (s, 3H), 3.72-3.64 (m, 3H), 3.56-3.46 (dd,

1H), 2.74-2.56 (m, 6H); ESMS ($C_{47}H_{48}N_2O_5$): calcd. 720.9, obsd. 721.4 [M+H]$^+$, 743.3

[M+Na]$^+$.

To a suspension of LiAlH$_4$ (0.211 g, 5.56 mmole) in THF (40 mL) cooled with ice bath

was added 1-{2-[N-benzyl-N-2-(4-benzyloxy-3-methoxycarbonylphenyl)-2-(R)-

20   hydroxyethyl]aminoethyl}-4-[N-(2-phenyl-2-(S)-hydroxyethyl)amino]benzene (2.0 g, 2.78

mmole) in THF (10 mL) under nitrogen atmosphere. The reaction mixture was warmed

slowly to rt and the stirring was continued for 5 h. The reaction was cooled to 0 °C, and

10% NaOH (0.5 mL) was slowly added. After 30 min., a thick gel formed. The gel was

diluted with THF (300 mL), filtered, and the solid mass was rinsed with THF (50 mL).

25   The filtrates were combined, and concentrated *in vacuo*, yielding an oily residue. The

residue was purified by flash silica column chromatography (2/1 hexane/EtOAc to 3%

MeOH in 1/1 hexane/EtOAc) to give 1-{2-[N-benzyl-N-2-(4-benzyloxy-3-

hydroxymethylphenyl)-2-(R)-hydroxyethyl]aminoethyl}-4-[N-(2-phenyl-2-(S)-

hydroxyethyl)amino]benzene as a colorless oil (1.28 g, 67%). H^1-NMR (CD$_3$OD, 299.96

30   MHz): δ (ppm) 7.4-7.0 (m, 22H), 6.85-6.82 (m, 3H), 6.63-6.60 (d, 2H), 5.02-4.94 (m, 3H),

4.66 (s, 2H), 4.59-4.54 (dd, 1H), 4.48-4.4 (d, 1H), 4.24-4.16 (d, 1H), 3.76-3.7 (d, 1H),
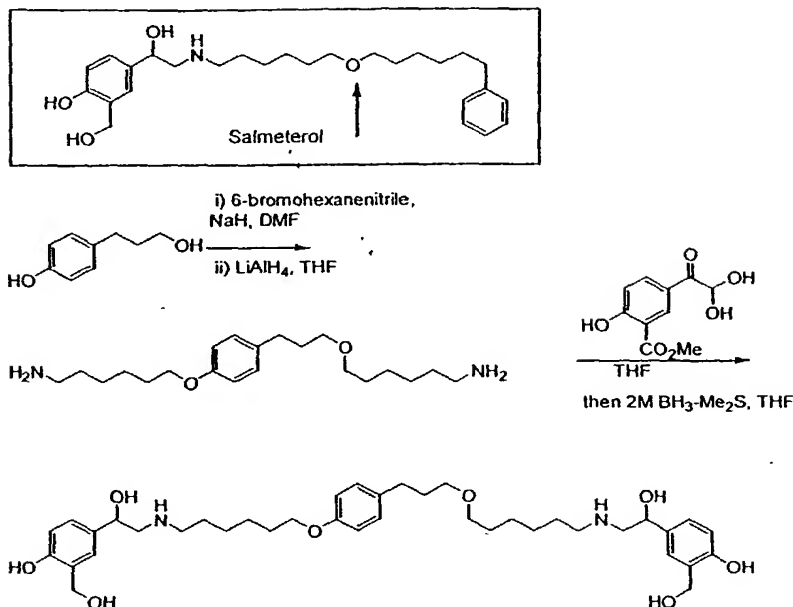
3.69-3.62 (dd, 1H), 3.58-3.52 (d, 1H), 3.50-3.44 (dd, 1H), 2.76-2.54 (m, 6H); ESMS ($C_{46}H_{48}N_2O_4$): calcd. 692.90, obsd. 693.5 [M+H]$^+$.

A solution of 1-{2-[N-benzyl-N-2-(4-benzyloxy-3-hydroxymethylphenyl)-2-(R)-hydroxyethyl]amino]ethyl}-4-[N-(2-phenyl-2-(S)-hydroxyethyl)amino]-benzene (1.28 g, 1.85 mmole) in EtOH (80 mL) was hydrogenated under H_2 (1 atm) with 10% Pd/C (0.6 g) for 36 h. After filtration and rinsing of the catalyst with EtOH (50 mL), the filtrates were combined, and evaporated *in vacuo*, yielding pale yellow foam which was dissolved in 10% MeCN/H_2O, and purified by reversed phase preparative liquid chromatography (10 to 30% MeCN/H_2O (containing 0.3% TFA) over 50 min; 30 mL/min; 254 nm) to give 1-{2-[N-2-(4-hydroxy-3-hydroxymethyl-phenyl)-2-(R)-hydroxyethyl]aminoethyl}-4-[N-(2-phenyl-2-(S)-hydroxyethyl)-amino]benzene as the TFA salt (0.6 g, 50%). Optical purity of 1-{2-[N-2-(4-hydroxy-3-hydroxymethylphenyl)-2-(R)-hydroxyethyl]aminoethyl}-4-[N-(2-phenyl-2-(S)-hydroxyethyl)amino]benzene which was analyzed with capillary electrophoresis by using a chiral medium, and estimated to be ~93%.

H^1-NMR (CD$_3$OD, 299.96 MHz): δ (ppm) 7.42-7.28 (m, 8H), 7.26-7.22 (d, 2H), 7.18-7.14 (dd, 1H), 6.80-6.77 (d, 1H), 4.88-4.82 (m, 2H), 4.65 (s, 2H), 3.5-3.43 (m, 2H), 3.29-3.26 (m, 2H), 3.19-4.14 (m, 2H), 3.06-3.0 (m, 2H); ESMS ($C_{25}H_{30}N_2O_4$): calcd. 422.5, obsd. 423.1 [M+H]$^+$, 445.4 [M+Na]$^+$,

EXAMPLE A61

i) 6-bromohexanenitrile, NaH, DMF

ii) LiAlH₄, THF

then 2M BH₃-Me₂S, THF

A solution of 3-(4-hydroxyphenyl)-1-propanol (2.0 g, 13.1 mmole) in DMF (5 mL)
was added to a solution of DMF (35 mL) containing NaH (1.31 g, 60% in mineral oil) at
5   0°C under nitrogen atmosphere. The reaction mixture was slowly warmed to 80 °C. After
stirring for 1 h at 80 °C, the reaction mixture was cooled to 0 °C, and 6-bromohexanenitrile
(5.78 g, 32.83 mmole) was added. The final mixture was re-heated to 80 °C, and stirred for
24 h. The reaction mixture was quenched with saturated NaCl solution (200 mL), and the
product was extracted with EtOAc (300 mL). The organic layer was washed with brine
10   solution, dried with Na₂SO₄, and evaporated to dryness, yielding a pale yellow solid.
Purification of the crude product by flash silica column chromatography: 4/1 to 1/1
hexane/EtOAc provided 6-{3-[4-(5-cyanopentyloxy)phenyl]propoxy}hexanenitrile in 30%
yield (1.33 g). R_f = 0.63 in 1/1 EtOAc/hexane. ¹H-NMR (CDCl₃, 299.96 MHz): δ (ppm)
7.09-7.07 (d, 2H), 6.81-6.78 (d, 2H), 3.96-3.92 (t, 2H), 3.42-3.37 (m, 4H), 2.64-2.58 (t,
15   2H), 2.39-2.32 (m, 4H), 1.87-1.52 (m, 14 H).

A solution of 6-{3-[4-(5-pentyloxy)phenyl]propoxy}hexanenitrile (1.33 g, 3.88 mmole)
in THF (10 mL) was added to a solution of LiAlH₄ (0.442 g, 11.65 mmole) in THF (50
mL) at 0 °C under nitrogen atmosphere. The reaction mixture was heated slowly to reflux,
and stirred for 2 h. The reaction mixture was cooled to 0 °C, and 10% NaOH solution (5
20   mL) was slowly added. After 30 min., the reaction mixture was filtered, and the collected

solids were washed with THF (100 mL). The filtrate was concentrated to yield a pale yellow oil which was purified by flash silica column chromatography: 5% MeOH/CH_2Cl_2 to 3% i-PrNH$_2$/20% MeOH/CH_2Cl_2 to give 6-{3-[4-(6-aminohexyloxy)-phenyl]propoxy}-hexylamine as a colorless oil (0.5 g, 37%).

5   To a suspension of α, α'-dihydroxy-4-hydroxy-3-methoxycarbonyl-acetophenone (0.3 g, 1.33 mmole), prepared as in Example A55, in THF (10 mL) was added a solution of the above compound (76 mg, 0.66 mmole) in THF (5 mL). The resulting suspension was stirred for 3 h at ambient temperature under nitrogen atmosphere, at which formation of an imine was completed judged by TLC analysis. After cooling of the resulting solution at ice

10   bath, an excess amount of 2M BH$_3$-Me$_2$S in hexane (4 mL, 8 mmole) was added to the previous solution. The resulting mixture was slowly warmed to rt and refluxed for 4 h under N$_2$ stream. After cooling the reaction mixture, MeOH (5 mL) was added to quench excess amount of 2M BH$_3$-Me$_2$S. After stirring for 30 min., the final solution (or cloudy solution) was evaporated *in vacuo*, yielding a pale brown solid. The solid was washed with

15   EtOAc/hexane (1/2; 20 mL), and dried. The crude product was dissolved in 50% MeCN/H$_2$O containing 0.5% TFA, and purified by prep-scale high performance liquid chromatography (HPLC) using a linear gradient (5% to 50% MeCN/H$_2$O over 50 min, 20 mL/min; detection at 254 nM). Fractions with UV absorption were analyzed by LC-MS to isolate *trans*-1,4-bis{N-[2-(4-hydroxy-3-hydroxymethyl-phenyl)-2-

20   hydroxyethyl]amino}cyclohexane. The crude product was purified by preparatory reversed phase HPLC: 10 to 40% MeCN/H$_2$O over 40 min; 20 mL/min; 254 nm. ESMS (C$_{39}$H$_{58}$N$_2$O$_8$): calcd. 682.8, obsd. 683.6 [M+H]$^+$, 797.5 [M+CF$_3$CO$_2$H]$^+$.

EXAMPLE A62
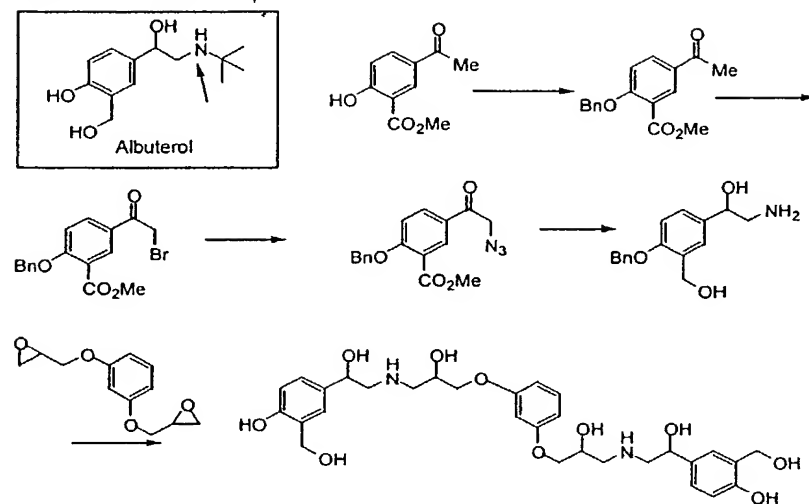
To a N_2-saturated solution of acetonitrile (300 mL) containing methyl 5-acetylsalicylate (20 g, 0.1 mole) and benzylbromide (13.5 mL, 0.11 mole) was added K_2CO_3 (28.5 g, 0.21 mole). The reaction mixture was stirred at 90°C for 5 h. After cooling, the reaction mixture was filtered, and the filtrate was concentrated, *in vacuo*, yielding a white solid which was susended in hexane (300 mL), and collected on BΠchner funnel to give methyl O-benzyl-5-acetylsalicylate as colorless to white crystals (28.1 g, 96%). $R_f = 0.69$ in 1/1 EtOAc/hexane. H^1-NMR (CDCl$_3$, 299.96 MHz): δ (ppm) 7.8.43-8.42 (d, 1H), 8.1-8.04 (dd, 1H), 7.5-7.28 (m, 5H), 7.08-7.04 (d, 1H), 5.27 (s, 2H), 3.93 (s, 3H), 2.58 (s, 3H).

To a solution of methyl O-benzyl-5-acetylsalicylate (14.15 g, 0.05 mole) in CHCl$_3$ (750 mL) was added bromine (2.70 mL, 0.052 mole). The reaction mixture was stirred at rt. While being stirred, the reaction mixture gradually turned from red-brown to colorless. The mixture was stirred for 2 h at rt, and quenched by adding brine solution (300mL). After shaking the mixture in a separatory funnel, organic layer was collected, washed with brine, and dried under Na_2SO_4. The organic solution was concentrated *in vacuo*, yielding white solid. It was washed with ether (200 mL). After drying in air, 15 g (83%) of methyl O-benzyl-5-(bromoacetyl)-salicylate was obtained. $R_f = 0.76$ in 1/1 EtOAc/hexane. H^1- NMR (CDCl$_3$, 299.96 MHz): δ (ppm) 8.48-8.46 (d, 1H), 8.14-8.08 (dd, 1H), 7.51-7.3 (m, 5H), 7.12-7.09 (d, 1H), 5.29 (s, 2H), 4.42 (s, 2H), 3.94 (s, 3H).

To a solution of DMF (60 mL) containing methyl O-benzyl-5-(bromoacetyl)-salicylate (7.08 g, 0.019 mole) was added NaN$_3$ (1.9 g, 0.029 mole). After stirring at rt for 24 h in

the dark, the mixture was diluted with EtOAc (200 mL), and washed with brine solution (3 x 200 mL) in a separatory funnel. The organic phase was dried under MgSO$_4$, and concentrated to afford pale red solid. It was purified by flash silica column chromatography: 10 to 50% EtOAc in hexane. The desired product methyl O-benzyl-5-(azidoacetyl)salicylate was obtained as white crystals (4.7 g, 74%). R$_f$ = 0.68 in 1/1 EtOAc/hexane. H^1-NMR (CDCl$_3$, 299.96 MHz): δ (ppm) 8.38-8.36 (d, 1H), 8.08-8.04 (dd, 1H), 7.5-7.3 (m, 5H), 7.12-7.09 (d, 1H), 5.29 (s, 2H), 4.53 (s, 2H), 3.94 (s, 3H).

To a gray suspension of LiAlH$_4$ (2.74 g, 0.072 mole) in THF (400 mL) cooled in ice bath was added methyl O-benzyl-5-(azidoacetyl)salicylate (4.7 g, 0.014 mole) under nitrogen atmosphere. The reaction mixture was stirred at 0°c for 1 h, and gradually warmed to rt. After stirring for 16 h at rt, the mixture was heated at 75°c for 3 h. The reaction mixture was cooled in ice bath, and quenched by slowly adding 10% NaOH (10 mL). After stirring for 1 h, precipitates were filtered, and rinsed with 5% MeOH in THF (200 mL). Filtrates were combined, and concentrated *in vacuo*, yielding pale yellow oily residue. The crude product was purified by flash silica column chromatography: 10% MeOH/CH$_2$Cl$_2$ to 5% i-PrNH$_2$ in 30% MeOH/CH$_2$Cl$_2$ to give 2-(4-benzyloxy-3-hydroxymethylphenyl)-2-hydroxyethylamine as a pale yellow solid (2.6 g, 66%). R$_f$ = 0.63 in 5% i-PrNH$_2$ in 30% MeOH/CH$_2$Cl$_2$. H^1-NMR (CD$_3$OD, 299.96 MHz): δ (ppm) 7.46-7.28 (m, 6H), 7.24-7.20 (dd, 1H), 7.0-6.96 (d, 1H), 5.11 (s, 2H), 4.70 (s, 2H), 4.65-4.60 (t, 1H), 2.83-2.81 (d, 2H); ESMS (C$_{16}$H$_{19}$N$_1$O$_3$): calcd. 273.3, obsd. 274.7 [M+H]$^+$, 547.3 [2M+H]$^+$.

To a solution of EtOH (15 mL) containing 2-(4-benzyloxy-3-hydroxymethylphenyl)-2-hydroxyethylamine (0.3 g, 1.1 mmole) was added resorcinol diglycidyl ether (0.122 g, 0.55 mmole) dissolved in EtOH (5 mL). The reaction mixture was refluxed for 20 h. After cooling down to rt, the reaction mixture was degassed with nitrogen and hydrogenated with 10% Pd/C (0.3 g, 10%) under H$_2$ (1 atm) atmosphere for 24 h. After filtration of the catalyst, the filtrate was concentrated to dryness, yielding a colorless oily residue which was purified by preparatory reversed phase HPLC (10 to 50% MeCN/H$_2$O over 40 min; 20 mL/min; 254 nm) to give bis{2-{2-(4-hydroxy-3-hydroxymethylphenyl)-2-hydroxy]-ethyamino}-2-hydroxyethoxy}benzene. ESMS (C$_{30}$H$_{40}$N$_2$O$_{10}$): calcd. 588.6, obsd. 589.4 [M+H]$^+$, 610.7 [M+Na]$^+$.
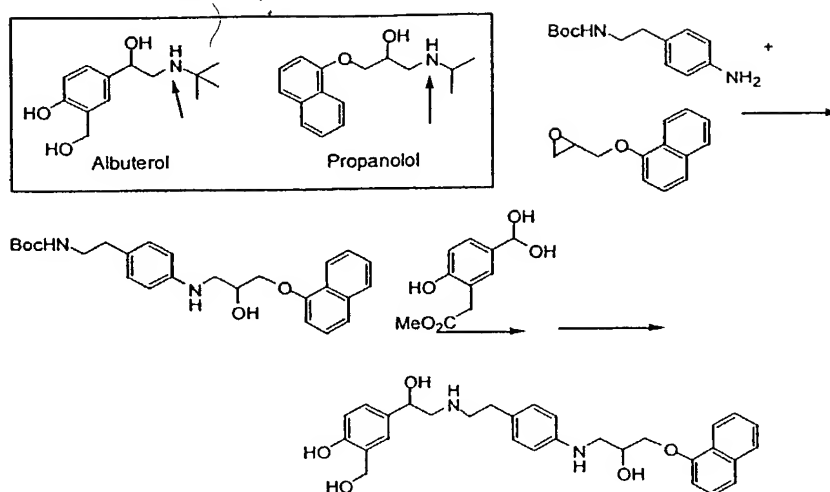
EXAMPLE A63

A solution of EtOH (50 mL) containing 4-(*N*-Boc-2-aminoethyl)aniline, prepared in Example A57, (0.4 g, 1.69 mmole) and 3-(1-naphthoxy)-1,2-epoxypropane (0.33 g, 1.65 mmole) was refluxed for 18 h, and concentrated *in vacuo* to dryness, yielding a pale yellow oil. It was dissolved in 10 mL of CH_2Cl_2, cooled in ice bath, and treated with TFA (5 mL). After stirring for 2 h at 0°C, the mixture was evaporated, yielding a pale red oil. It was dissolved in 30% aqueous acetonitrile, and purified by preparatory HPLC: 10 to 30% MeCN/H_2O over 30 min; 20 mL/min; 254 nm. The product was obtained as colorless oil (260 mg; TFA salt). H^1-NMR (CD$_3$OD, 299.96 MHz): _ (ppm) 8.88-8.25 (dd, 1H), 7.82-7.79 (dd, 1H), 7.51-7.42 (m, 3H), 7.39-7.38 (d, 1H), 7.33-7.30 (d, 2H), 7.25-7.23 (d, 2H), 6.91-6.89 (d, 1H), 4.37-4.31 (m, 1H), 4.22-4.19 (m, 2H), 3.69-3.63 (dd, 1H), 3.67-3.54 (dd, 1H), 3.17-3.11 (t, 2H), 2.96-2.91 (t, 2H); ESMS ($C_{21}H_{24}N_2O_2$): calcd. 336.4, obsd. 337.5 [M+H]$^+$, 359.6 [M+Na]$^+$, 673.4 [2M+H]$^+$.

To a solution of the above compound (0.13 g, 0.023 mmole; TFA salt) in 5 mL of MeOH was added 1.0 M NaOH (1.0 M, 0.46 mL). After homogeneous mixing, the solution was evaporated to dryness. The residue was dissolved in THF (10 mL), followed by addition of glyoxal (52 mg; 0.023 mmole). The resulting suspension was stirred for 4 h at ambient temperature under nitrogen atmosphere. After cooling of the resulting solution in ice bath, an excess amount of 2M BH$_3$-Me$_2$S in THF (3 mL; 6 mmole) was added to the

previous reaction solution. The resulting mixture was slowly warmed to rt, and refluxed for 4 h under N_2 stream.

After cooling of the hot solution, 5 mL of MeOH was added to the cooled mixture to quench the reaction mixture under nitrogen atmosphere. After stirring 30 min at rt, the

5   final solution was evaporated *in vacuo*, yielding a pale brown solid. It was washed with EtOAc/hexane (1/2; 20 mL), and dried. The crude product was dissolved in 50% MeCN/H_2O containing 0.5% TFA, and purified by prep-scale high performance liquid chromatography (HPLC) using a linear gradient (5% to 50% MeCN/H_2O over 50 min, 20 mL/min; detection at 254 nM). Fractions with UV absorption were analyzed by LC-MS to

10   locate the desired product 1-{2-[N-2-(4-hydroxy-3-hydroxy-methylphenyl)-2-hydroxyethyl]amino]-ethyl}-4-[N-(2-napth-1-yloxymethyl-2-hydroxy-ethyl)amino]benzene. ESMS ($C_{30}H_{34}N_2O_5$): calcd. 502.6, obsd. 503.2 [M+H]$^+$, 525.6 [M+Na]$^+$.

EXAMPLE A64
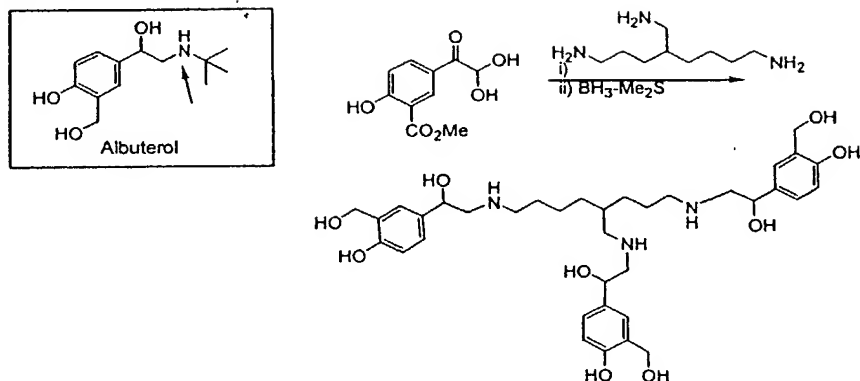
To a suspension of α,α'-dihydroxy-4-hydroxy-3-methoxycarbonyl-acetophenone prepared as in Example A55 (0.45 g, 1.99 mmol) in tetrahydrofuran (15 mL) was added a

5   solution of 4-(aminomethyl)-1,8-octadiamine (115 mg, 0.66 mmol) in tetrahydrofuran (5 mL). The resulting suspension was stirred for 12 h at ambient temperature under nitrogen atmosphere. After cooling of the resulting solution in ice bath an excess amount of 2 M BH$_3$-Me$_2$S in hexane (6 mL, 12 mmol) was added. The resulting mixture was slowly warmed to rt, and refluxed for 6 h under nitrogen atmosphere. After cooling, the reaction

10 mixture was quenched with methanol (5 mL). The resulting solution was stirred at rt for 30 min., and then concentrated in vacuo to give a pale brown solid. The solid was washed with ethyl acetate :hexane mixture (1:2) and then dried. The crude product was dissolved in 50% acetonitrile/water containing 0.5% TFA and purified by HPLC using a linear gradient (5% to 50% MeCN/H$_2$O over 50 min., 20 mL/min.; detection at 254 nM).

15 Fractions with UV absorption was analyzed by LC-MS to locate the desired product. ESMS (C$_{36}$H$_{53}$N$_3$O$_9$): Calcd. 671.8; Obsd. 671.7.

While the present invention has been described with reference to the specific embodiments thereof, it should be understood by those skilled in the art that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. In addition, many modifications may be made to adapt a particular situation, material, composition of matter, process, process step or steps, to the objective spirit and scope of the present invention. All such modifications are intended to be within the scope of the claims appended hereto.